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Is Local Behaviour in Salmon Heritable?

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ABSTRACT

Fry from Atlantic salmon taken in Chaleur bay, near the mouth of the Restigouche river, N.B., where the salmon enter early in the summer and mainly as two-sea-year and three-sea-year fish, were planted in 1932 in the East branch (without salmon) of the Apple river at the head of the bay of Fundy. In this region the local salmon enter only in the autumn and nearly all as one-sea-year fish (grilse). The Restigouche fish as parr grew more rapidly than the local fish, corresponding with the less crowded conditions in the East branch. The smolts were marked by removal of the adipose fin when descending to the sea in 1934. Their descent occurred from May 12 to June 20, being markedly accelerated by rainfall. Traps were placed on both branches in 1935, giving in the autumn 92 marked grilse entering the East branch and 6 the South. The traps in 1936 gave in the autumn, 5 marked two-sea-year salmon entering the East branch and 1 the South. No difference in appearance or behaviour was observable between these and the local salmon of the South branch.

INTRODUCTION

It has long been known and commented upon that the salmon (*Salmo salar*) of the Atlantic coasts of Europe and North America shows local pecularities in behaviour as to the time of the year and the age (as shown by size) at which it returns to fresh water from the sea. Particular rivers or even branches of a river may be characterized by the times of the year when salmon enter them and by the proportions among the virgin fish of grilse (growth of one year or somewhat more in the sea), ordinary salmon (growth of two years or somewhat more in the sea,) and big salmon (growth of three years or more in the sea). These facts have been commonly interpreted as meaning that there are more or less distinct races in the various rivers, characterized by such behaviour and also by certain body proportions and other details of structure. This conception has been generally accepted and very much work has in recent years been done in describing the peculiarities of the salmon of individual rivers, by making use of the scales of the fish for determination of the life-history of each individual.

Even for one river the salmon may come in from the sea at quite different seasons and these "runs" may differ considerably in character, such as size and body proportions. The theory has been extended to fit these facts by the supposition that there are more or less distinct early-running and late-running races in the one river.

Menzies (1925a, pp. 192-205) deals with the possibility of local and seasonal "races" in *Salmo salar*, stating the doubtful character of the evidence, but finally favouring the idea of a separate homogeneous race of salmon in each district. Rich and Holmes (1928) as a result of marking experiments with spring-running and fall-running chinook salmon (*Oncorhynchus tschawytscha*) report that the returns from marked fry and fingerlings show that the young from spring-running fish, when reared and liberated in tributaries normally inhabited by fall-running fish, return as adults predominantly in the spring, and that the converse also holds good. Calderwood (1930a, pp. 39-51) quotes these results and considers that the proof applies equally well to the Atlantic salmon, but this does not necessarily follow. Even within the one species proof or disproof that a certain character differentiates races in certain instances does not rule out the possibility of the opposite being true elsewhere. A character may vary with environment, or vary with the race, when the environment is the same.

The early running and the large fish (which two features are generally associated) are of most value to the fishermen. It has been natural, therefore, that the latter have wished to have the theory of races put to practical use in two ways: first by having the early and large fish used in fish cultural practice so that their numbers might be maintained and increased, and, second, by having the young from rivers with early-running and large salmon planted in those rivers where the local fish are late-running and small. To secure the early and large fish and to hold them until spawning time about the first of November was, therefore, undertaken by the Fish Culture Branch of the Department of Fisheries of Canada and proved to be a difficult and costly practice. This made it desirable to ascertain whether the practice had any real justification.

Late-running fish in the Miramichi river, New Brunswick, after having been spawned, were tagged and liberated. Of those recaptured in the river after one or two years and presumably ready to spawn again, six out of sixteen were taken before August 15, one as early as June 1 (Rodd 1924, 1925, 1926, 1927), which for that river is quite early. This definitely raised doubts as to the distinctness of the early-running and late-running habits in individual fish. Calderwood (1930b, p. 16), however, has argued that the seasonal habit persists in individual fish, making use of the fact that of all the grilse (which run in summer) marked in Scotland, not one when recaptured as a salmon was taken in the spring.

He has also argued that the heavy netting of the salmon in summer and autumn on many parts of the coast of Scotland has caused the late-running grilse to decline and the spring salmon to increase, and that large catches of salmon in the autumn and summer in the Tweed district resulted in the Tweed changing in twenty-five years' time from a late river into an early river. He states "if we do not accept the premise (the existence of early and late runs as separate local races of salmon) I am unable to account for the spring run in the Tweed in any other way" (1930b, p. 17). However, Huntsman (1931, p. 94) concludes for the Miramichi river "that man's experiment lasting for more than eighty years in restricting fishing to the early run and in encouraging reproduction (natural as well as artificial) of the late run has succeeded neither in materially reducing the early run nor in materially increasing the late run."

With such conflicting views, it seemed desirable after a study of the local behaviour of the salmon on different parts of the Canadian coast to devise and carry out the most crucial test possible of the existence of races of salmon differing as to season and age at return—a test that would determine whether or not such peculiarity in behaviour is inherited.

LOCAL BEHAVIOUR IN CANADIAN WATERS

As a background for the proposed experiment it seemed desirable to have knowledge of the local variations in salmon behaviour. While the salmon is found from the New England states to northern Labrador, Canadian waters cover its whole present range in latitude, except for northern Labrador. The life-history study by Lindsay and Thompson (1932) of the Newfoundland and

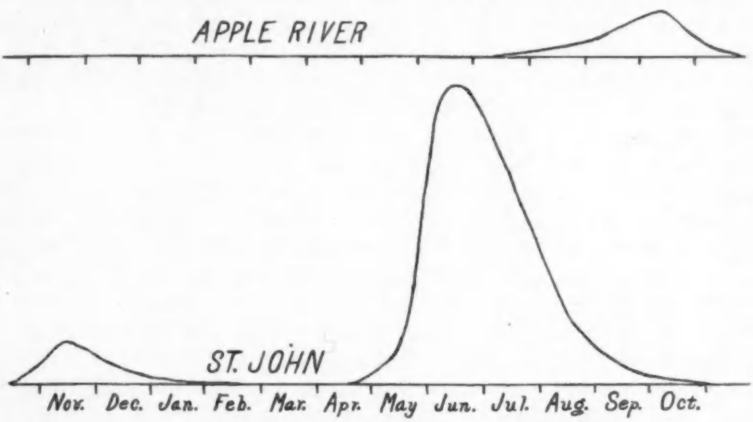

FIGURE 1. Salmon runs in Fundy rivers; spawning in October.

Labrador salmon shows similar variety in behaviour to that occurring in Canadian salmon. The life-history of the latter has been studied by Menzies (1925b), Calderwood (1927), Phelps and Belding (1930), Huntsman (1931) and Blair (1935). Although only a small part of the many rivers and coastal districts has been so covered, enough is known to give a fairly clear picture of the variety that is to be found.

For the Saint John river of southern New Brunswick, there is good evidence that toward the end of May after a quiescent winter season the salmon start to move both in the estuary, where quite a number pass the winter, and in the sea outside the river mouth. From that time until the next January the fish enter the river from time to time (figure 1). Those of a given smolt year-class (descending as smolts in a given year) begin to be caught and enter the river as grilse in June of the following year and increase in size and number with progress of the season. These are practically all males, spawn that autumn, and on the whole would be called a late run. From October to January there is a moderate

run of fish, quite immature females that will not spawn until the following autumn (Huntsman 1933). They average about 9 lb. (4 kg.) in weight and are thus intermediate between the grilse and the ordinary spring salmon. Most of them remain in the estuary during the winter and ascend the river above the head of tide only in the following spring. The main mass of the fish enter the river in their third sea year, starting late in May, and increasing in size with the progress of the season, and in number up to July. Females greatly predominate at first. Very few fish remain for another year to become big salmon.

In relation to spawning the earliest running fish (October to January) are females and the latest running fish are the male grilse, while the main lot runs during an intermediate season (May to August). It is difficult to understand how there can be an early running race and a late-running race, since both sexes are required for each race.

Perley (1852, pp. 142, 149, 153, 156) reports that the characteristic salmon around the upper end of the bay of Fundy are "fidlers" or grilse. In the autumn of 1931 we canvassed the fishermen all around the bay and obtained full confirmation of this difference between the upper (inner) and lower (outer) parts of the bay, the latter being represented by the Saint John river.

In Chignecto bay and tributary waters, the salmon are taken in weirs, drift nets and set-nets. They range in weight from 2 to 16 lb. (0.9 to 7.25 kg.), do not appear before June, become more numerous with the advance of the season and enter the rivers in the autumn (as for Apple river in figure 1). Fishery Inspector Bruce Barnes obtained for us in 1931 data and scales from 34 salmon taken by gill nets in Cumberland basin and Shepody bay from August 27 to October 8, and from 2 salmon taken in the Petitcodiac river on October 16. Of these 20 were considered males and 16 females. The scales showed that 33 were virgin fish, 32 of them grilse and 1 a two-sea-year fish. All of the remaining 13 had previously spawned as grilse. One female had spawned three times, was preparing for a fourth spawning and yet was only 68½ cm. in length and 2.4 kg. in weight! The heaviest (the two-sea-year virgin) weighed only 4.5 kg. and measured 73½ cm. One had spent one year in the river before reaching the smolt stage, 32 had spent two years and 3 had spent three years. These fish are very definitely characterized by having a two-year river life, and four years from spawning to spawning, and by entering the rivers only late in the season.

In Minas basin and tributary waters the salmon are taken in weirs, drift nets and set nets. They range in weight from 3 to 30 lb. (1 to 14 kg.), appear in June and increase in numbers through the season both out in the bay and in the tidal waters. The larger ones, 8 to 10 lb. (3½ to 4½ kg.), appear first, and the great majority are grilse, 3 to 6 lb. (1 to 2¾ kg.). They enter the rivers in the autumn. Examination of a small number of individuals showed that both females and males spawn as grilse, and that most of the fish spend two years in the river before becoming smolts (Huntsman 1931, p. 19). The statistics indicate good years tending to repeat at two-year and four-year intervals, corresponding to two years in the river and four years from spawning to spawning.

The salmon of the outer coast of Nova Scotia have been little investigated, but, as reported, they are on the whole similar to those of the Saint John river

in having both grilse and small salmon, with very few big salmon. In the south-western part some of the fish are said to enter the rivers during the winter months, which corresponds with the fact that cod and haddock are also available at that season in the same region, and with the fact that the temperature of the sea is higher there than elsewhere. In the gulf of St. Lawrence on the inner coast of Cape Breton island, the salmon begin to move only about the beginning of June, and they continue to be available till the autumn. All sizes occur at all times, there being mostly small salmon, a fair proportion of big salmon and very occasional grilse.

In the western part of the gulf there is a somewhat graded series going northward along the east coast of New Brunswick and Gaspesia. At the south there are grilse and small salmon. For the Miramichi, Blair (1935, p. 160) gives grilse as forming 60 per cent of the fish taken in a net in the estuary in 1931, and two-sea-year fish forming 85 per cent of the fish larger than grilse. Going northward the grilse diminish in numbers, while big salmon become more abundant. The climax is reached in Chaleur bay, the Cascapedia river on the north side being without grilse and with nearly ten times as many big salmon as small salmon (Calderwood 1927). The great majority of the fish remain in the river for three or four years before becoming smolts. Study of the statistics of the annual catches in this region of Gaspesia reveals evidence of better catches every four and eight years, corresponding with four years of freshwater life and eight years from spawning to spawning (Huntsman 1931, pp. 72-75). This is twice the length of life-history of the salmon at the head of the bay of Fundy.

Available information indicates that the north shore of the gulf has intermediate conditions, with nothing so extreme as either the Cascapedia fish on the one hand or as those of the upper end of the bay of Fundy on the other.

RESTIGOUCHE VERSUS APPLE RIVER SALMON

For a crucial test of there being distinct races differing in behaviour, it would have been desirable to have compared Cascapedia salmon with the salmon of Chignecto bay, these showing the greatest contrast. Unfortunately neither of these is used as a source of eggs for fish cultural purposes. However, other Chaleur bay salmon than Cascapedia are so used, namely Restigouche salmon, and their fry have been available for planting side by side with the Chignecto bay salmon for an accurate comparison of their behaviour under the same or closely similar conditions. The stream selected for this planting was Apple river at the upper end of the bay of Fundy, a district distinctly characterized by having late, small fish.

Fish that are considered to be *Restigouche* salmon are taken by nets outside the mouth of that river at the head of Chaleur bay on the south side, and those to provide eggs for the Restigouche hatchery are obtained near New Mills where the retaining pond is located. Just as for any fish whose individual histories from birth are not precisely known, there is no certainty that these fish are from the Restigouche river. However, seeing that they are in the course taken by the water of that river, before its influence is much lessened, the presumption is

strong that they are Restigouche river fish. The Restigouche county catch to which these belong shows for the ten-year period, 1926 to 1935, the following average quantities in cwt. (220.5 kg.) for each month: May, 136; June, 3,422; July, 1,462; and August, 190. Salmon enter the Restigouche river early in the season and these salmon are taken early, but it cannot be stated when they would enter the river. The salmon which furnished the fry for the experiment were taken in May, June and July of 1931 and were held till spawning time, which was from October 20 to November 10 (Rodd 1932, p. 13). About half (408) of those taken in 1930 for spawning, of which about 44 per cent were males, were measured and weighed after being stript. From partial examination of the scales from these fish, and from the lengths of all, it is believed that but a small percentage had previously spawned and that, of the virgin fish, approximately a third were big salmon, that is, had been three years in the sea, being upwards of 84 cm. in length. The nets do not take the grilse, but Dr. D. L. Belding informs us that grilse formed 10.7 per cent of the total angling catch in the Restigouche river from 1881 to 1930. The Chaleur bay salmon used for the experiment may, therefore, be considered normally to consist of 10 per cent grilse, 60 per cent two-sea-year fish, and 30 per cent three-sea-year fish, although actually no grilse were used for spawning.

The great majority of the fish had spent three years in the river before going to sea, although some had spent four or two years. This is in sufficiently good agreement with the life-history analysis of Restigouche salmon for the year 1930, given by Phelps and Belding (1930, p. 23),—54.6% two-sea-year, 41.3% three-sea-year, and 4.1% four-sea-year; 17.4% two-river-year, 76.8% three-river-year, 5.8% four-river-year, among the virgin fish. However, the previously spawned fish amounted to 31% as compared with a negligible amount (1 in 18) among those taken at New Mills for fish cultural purposes. From these facts we may safely conclude that Restigouche salmon are characterized by spending for the most part three years in the river and two or three years in the sea.

The *Apple river* salmon were examined in 1931, as well as in subsequent years. As expected from the location of the river, the salmon proved to be spending two years in the river and something over a year in the sea, returning to the river as grilse in the autumn. Of 3 fish taken in the estuary on August 18 and 22, and 60 fish taken in the South branch from September 11 to October 15, 1931, 55 had been two years in the river, 6 had been three years and 2 were doubtful. Of these same 63 fish, 59 were virgin, 52 being grilse and 7 two sea-year fish; all of the others (4) had spawned previously, one of them having spawned twice and yet only 65 cm. in length. Of the grilse and those that had spawned as grilse 36 were females and 20 were males. All the two-sea-year fish were females.

Confirming the analysis as to length of river life, 21 large parr taken one and a half miles (2.4 km.) up the river on September 12, 13 females and 8 males, one (14 cm. long) was in its third year, but all the others (9.6 to 12.5 cm. long) were in their second year. It would be expected that toward the limit of the distribution of the young upstream the proportion in the third year would be greater. All the male parr became sexually mature in the autumn and produced

milt, while their scales did not come off so easily and showed slight absorption on the sides.

In November (9 to 23) after spawning was over, 54 kelts were taken and of these 33 were grilse, 9 were two-sea-year fish, 11 had previously spawned as grilse (one had spawned twice), and one had spawned previously as a two-sea-year fish.

On the whole it has been found that over 90 per cent of the fish spawn as grilse and the remainder as two-sea-year fish, while about 90 per cent have two years' growth in the river and the remainder three, except a very occasional one with four.

To sum up, the Restigouche salmon are classed as early running, and characteristically spend three years in the river and two or three years in the sea, making six or seven years from spawning to spawning. The Apple river salmon are classed as late running, and characteristically spend two years in the river and return as grilse, making four years from spawning to spawning.

CONDITIONS IN APPLE RIVER

The river in which the two types have been compared under closely similar conditions is in Cumberland county, Nova Scotia, and empties into Chignecto bay at the head of the bay of Fundy. The river consists of two branches, East and South, with separate tidal estuaries, which unite into a common part before entering the bay (figure 2). The branches may be regarded as separate streams, and these are very similar in flow, temperature and other characters. For some fifty or more years there was a dam at the head of tide on the East branch and this had formed a barrier to any spawning run of salmon into that branch. Before the building of the dam on the East branch it had been used by the salmon as a spawning stream. For ten years previous to our first visit there in 1931 the dam had been out, but the salmon had not become re-established in the stream, although trout (*Salvelinus fontinalis*) were abundant. We made numerous inquiries of anglers and residents concerning the matter and all reported that, at least for many years, no adult salmon had been known to ascend this branch. The anglers said that they had never taken salmon parr or smolts from this stream. There had been no dam forming a barrier on the South branch and it had been used regularly by the salmon as a spawning stream. During the run, salmon were seen in this stream and anglers reported the taking of many salmon parr and smolts. We made numerous seine hauls in both branches and caught many young salmon of all stages in the South branch, but throughout the summer of 1931 we failed to find any young salmon in the East branch. From our inquiries and observations we concluded that if young salmon were present in the East branch they were very scarce. However, in the spring of 1932 when thousands of smolts were present in the South branch just above the head of tide as well as in the estuary where they could be taken in large numbers, we captured three smolts just above the head of tide in the East branch. Since all our evidence had indicated the absence of salmon in this branch, we thought that these fish might have ascended this stream from the common estuary. Upward

migration of smolts in this stream has been observed as is described on p. 12 under "Behaviour during migration".

RESTIGOUCHE SALMON IN APPLE RIVER

Planting the Fry

A request was made to the Department of Fisheries that fry from early-run salmon from the Restigouche river be supplied for planting in the East branch of Apple river. On July 13, 1932, a shipment of approximately 25,000 fry with the yolk sacs just absorbed, arrived from the Flatlands hatchery on the Restigouche river, N.B. Previous to their arrival we had constructed a large floating

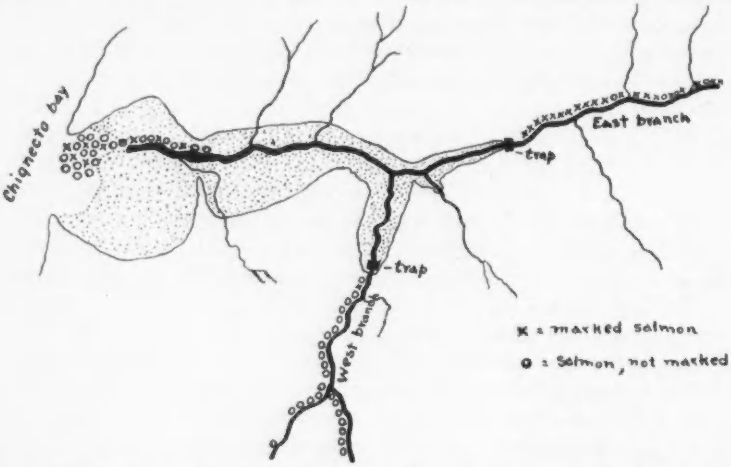

Figure 2. Map of Apple river showing the main stream and lower part of the two branches. "West" branch should be "South" branch. The extent of the tidal flats is indicated by stippled areas. The known positions of marked "Restigouche" (x) and unmarked local (o) grilse are shown where they were first in the estuary and later in the branches where they were after migration.

screen pound in which to retain the fry in the stream while distributions were being made along the stream. After a day's transportation by truck these fry were inactive, due no doubt to their becoming fatigued by the motion of the water in the cans during the journey. When placed in the pound they drifted with the current and collected near the lower screen and were but slightly sensitive to tactile stimuli. The following day their reactions were quite different. They were congregated near the upper screen and reacted readily to tactile stimuli or even to quick movements made above the water. They were retained in the pound for more than 24 hours so that they might fully recover from the effect of the journey and also become somewhat acclimated to the water of the river before being liberated.

On July 14 and 15 fry were carried up the river and liberated in small lots among the stones in the shallow water along the rapids. When a moderate

number of fry was poured from the distributing pail, some of them settled to the bottom and took positions among the stones, while others darted swiftly, like frightened native fry, up or down stream or away from the shore, where they soon took up positions among the stones. Within a few minutes after planting it was observed that the fry had begun feeding and no difference could be detected between their movements and the feeding movements of naturally hatched fry. After they had been liberated for a few hours, it was necessary in making observations on their feeding and other movements, to approach them with the same caution as would be required when making such observations on the native fry of a stream.

The Young in the River

No fry were planted on two rapids near the middle of the area of planting, and throughout the first summer no fry were observed on these rapids, indicating that salmon fry have little tendency to migrate through a pool from one rapid to another. Above the area of planting there was a long rapid and during the summer some of the fry planted just below it migrated as far as its head.

FIRST SUMMER

Throughout the summer bi-monthly samples of these fry as well as of native fry from the other branch were seined from the streams for measurements and for stomach analyses. It was found that they were feeding largely upon chironomid larvae and ephemerid nymphs (White 1936a, p. 502).

During their first summer in the stream these fry made a better relative growth than the native fry of the South branch, which had emerged from the gravel with yolk sacs absorbed a month before the arrival of the Restigouche fry (White 1933, p. 43).

Apple river salmon are mostly grilse which produce smaller eggs than those from the larger salmon such as are taken in the Restigouche. At the time the Restigouche fry were received they were smaller than the native Apple river fry which had been feeding for a month, still they were larger than the latter had been at the time of their emergence from the gravel. The more rapid growth of the Restigouche fry may have been due to better feeding conditions in the East branch where there was a lower concentration of fry or to further utilization of yolk fat which is normally stored in their fat reservoirs during yolk absorption.

By mid-October when observations were discontinued for the season their feeding had declined and some of the male fry which had attained a length of 7 cm. or more were sexually mature. Our observations indicated that there had been a good survival of the planted fry, as numbers of them could be seen in direct observation along the stream or easily captured with a small hand seine throughout the area where they had been planted.

SECOND YEAR

The following summer (1933) observations on the planted fish were continued (White 1934, p. 43). On our arrival on the East branch in late May we

found that most of the yearling parr were among the stones or in small pockets on the rapids. As the season progressed more parr, especially the larger ones, were found in the larger pools of the stream, although many apparently remained throughout the summer in favourable places on the rapids. During the summer they extended their range, a few being observed several miles (1 mile equals 1.6 km.) above the area of planting. Bi-monthly samples of the parr were seined from this stream and also samples of native parr from the South branch. The Restigouche fish continued to make a better growth than the native parr of the South Branch. The growth of the parr of each stream during the second summer is shown in figure 3. Since the measurements were of comparatively small numbers of parr and these from both rapid and pool habitats, their averages for the various samples may be expected to diverge rather widely from the curves

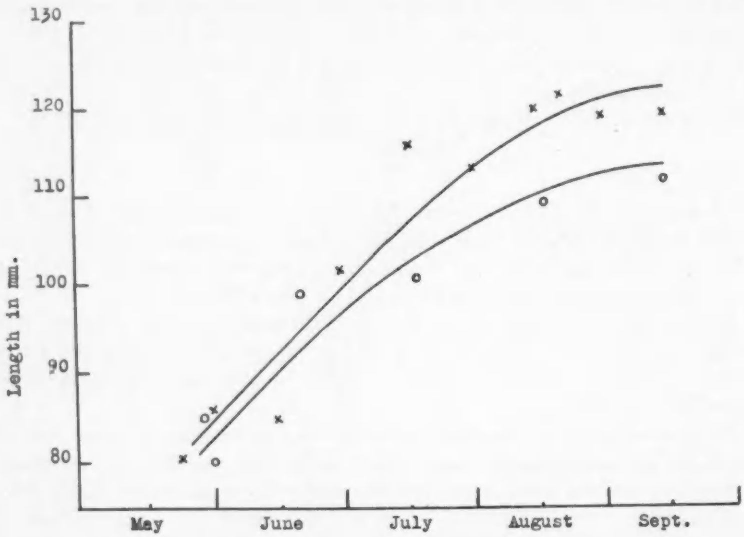

FIGURE 3. Growth of parr in East (x) and South (o) branches of Apple river in 1933.

representing the true course of the average growth. The growth curves show that growth is rapid during June and July and part of August, but falls off during late August and September. Food analyses of the young salmon show relatively small amounts present in their stomachs during September and October, and, in correspondence with this, figure 3 shows a decided slackening in growth during September. Moreover the scales of parr taken during September show that the so-called winter band is well completed. The parr by this time had attained an average length of 122 mm. and the male parr were sexually mature.

During late August of 1933 we attempted to ascertain the survival of the parr up to that time. Seinings were made along the stream, and parr were marked by the removal of the adipose fin. On re-seining portions of the same areas, approximately one-quarter of the marked fish were recaptured, which

shows our seining to have been about 25 per cent efficient. By this means we estimated that the surviving parr totalled about 7,400. Such calculations are subject to considerable error, but they do show that there had been a considerable loss among the fry. Since there was an abundance of food in the stream there was no danger of loss of fry from lack of food nor was there any evidence that disease was a factor. The only other factor which might cause loss seemed to be enemies, potential ones being trout (*S. fontinalis*), eels (*Anguilla rostrata*), fish-eating birds and anglers. Of these the fish-eating birds, especially the kingfisher (*Megaceryle alcyon*), were considered the most serious. There was also evidence that some loss had been caused by eels.

Descent of the Smolts

THE TRAP

In order to capture the smolts on their descent to the sea we started construction of a smolt trap on our arrival at Apple river on May 1, 1934, but, owing to high water, it was not finally completed until May 11. The barrier was composed of a series of slat racks with half-inch (1.27 cm.) spaces and was held in place by a heavily-ballasted framework of logs. The two wings of the barrier were at an angle to form a V, with the apex down stream and with the pound fitted in an opening at the apex. The entrance to the pound was of the conventional V-shape common to many types of fish traps. During the course of the experiment it was found necessary to narrow and lengthen the lead into the pound to make the trap more efficient in retaining the smolts on their first entrance. The barrier was placed across the stream at the down-stream end of the lowermost pool unaffected by the tide, and the pound occupied part of the lower end of this pool.

THE RUN

On our arrival we found no evidence of the smolt run having started. Few of the parr were as yet transforming to the smolt stage and even these were found far up the streams. There was no concentration of transforming fish in the lower pools. The stream was still in the spring high-water state, but from day to day it was dropping steadily. After the trap was placed examination of the stream below the trap and of the tidal waters for some distance down stream indicated the presence of only a few scattered individuals in the fresh water at the head of tide. In the pool above the trap there was a small school of less than 100 fish. Since the trap was just above the influence of tidal water, the records of the daily catches do not indicate the migration of the smolts to the sea, but only their migration down stream. Many of the incomplete smolts taken in the trap were probably fish caught while milling about the deep pool in the lower end of which the trap was situated, but at other times there was a definite down stream migration. This latter tendency was particularly noticeable as the season advanced, when large schools of smolts were present in the lower pools of the stream.

The first incomplete smolts were taken in the trap on May 12 and with certain fluctuations, mostly correlated with weather conditions, there was a rapid increase in the number toward the end of May, but during the first week in June the general trend became downward. The decline continued until June 20, when during a two-foot (61 cm.) rise of the stream the final run occurred.

A total of 3,252 smolts were taken in the trap and of these 11 were three-winter smolts of unknown origin (immigrants from South branch?) while 3,241 were two-winter smolts and presumably the survivors from the fry that had been planted. This number of smolts would give a survival of 13 per cent of the planted fry. The few parr which may have remained in the stream until the next year, of which we found no evidence, and those which were immediately below the trap when it was placed in position, would not materially alter this percentage.

BEHAVIOUR DURING MIGRATION

In the early part of May the incomplete smolts were found scattered throughout the length of the range occupied by the parr. As the season progressed and the smolt condition became more apparent they began to collect in the lower pools of the stream. They would form into a large school in the pool above the trap (the largest pool in the lower non-tidal part of the stream) and every night a few individuals would enter the trap. But on rainy nights, especially when the stream was rising, most of the fish in this pool would run downstream into the trap. At dusk the smolts could be seen entering the trap in small schools, but after dark there was no evidence of schooling and they entered the trap singly. All the smolts which we observed entering swam downstream into the trap. After being marked and liberated below the trap they went downstream and were found collected in a large school in the uppermost tidal pool which only the higher tides affected. Whether or not they had been downstream in the brackish water and had returned to this pool we are unable to state, but, from time to time, large numbers of them would reascend a swift rapid and attempt to pass the barrier or would collect in the still water below it.

Throughout the period of migration the smolts were feeding voraciously and would readily take a baited hook or artificial flies. On the warmer evenings during their stay at the head of tide, nearly a month for many of them, they could be seen jumping after chironomids which were hovering above the water. The smolts made a rapid growth during their transformation. This growth was indicated both by increase in size of the trapped smolts as the season progressed and also by the growth on the scales. Their growth is indicated in figure 4.

On June 8 we fished the estuary but took no smolts nor did we see any rising to the surface. From this observation and the fact that most of the fish released below the trap were congregated in the pool at the head of tide, we concluded that few of them had gone out to sea. However, on June 11 we found that the smolts had left the pool at the head of tide and June 12 many were in the brackish water pools a half-mile (0.8 km.) below. On June 13 no further trace of these smolts could be found.

After June 13, when most of the smolts had transformed, although small schools of them collected in the pool at the head of tide they did not remain long

but soon passed down into tidal water and disappeared. Although we have made seinings in the lower estuary soon after the disappearance of the smolts from the upper part we have failed to find them. Apparently they go directly out to sea. Calderwood reports that they pass out to sea during the ebb tide (1908, p. 20) and at the Margaree river, Cape Breton island, where the water is clear, we have observed their seaward migration during ebb tide. Throughout the trapping of the smolts on the East branch observations were made also on those of the South branch, which showed similar behaviour.

In figure 5 are shown the daily catches of smolts, and also the temperatures taken at 10.00 a.m. and 10.00 p.m. It will be noted that, excepting for the rise

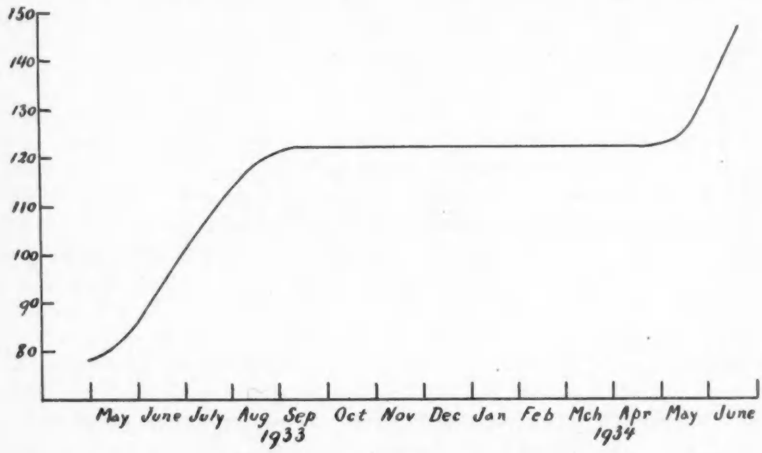

FIGURE 4. Growth of young salmon in East branch of Apple river during latter part of parr life and until departure for the sea as smolts.

of temperature during the first part of the run, coincident with the advance of the season, there is no correlation between temperature and the downstream migration. However, the occurrence of rain, especially enough to cause a small rise in the stream level, was accompanied by a sharp rise in the numbers entering the trap, as shown on May 30, June 4, June 10, and June 20. A rise in the stream level of one or two inches (2½ to 5 cm.) was sufficient to cause a definite downstream migration, but an increase in the migration did not occur with a further increase in the flow.

MARKING

Throughout the run the smolts were removed from the pound at 10.00 a.m. and 10.00 p.m. and more frequently when the run was especially heavy. Each time the trap was emptied the entrance to the pound was closed and the smolts dipped out in a net having a soft fine-meshed webbing. The complete adipose fin was shaved off each fish before it was released below the trap. At each

closing of the trap the several fish from a random sample were measured in a small V-shaped measuring trough and scale samples were saved. There have been objections to the use of the removal of the adipose fin as a method of marking since it has been claimed that this fin is regenerated and also that this mark is found as a natural mutilation. When this fin is shaved off close to the body there is no regeneration and it leaves a clean scar such as we have never observed in any natural mutilation.

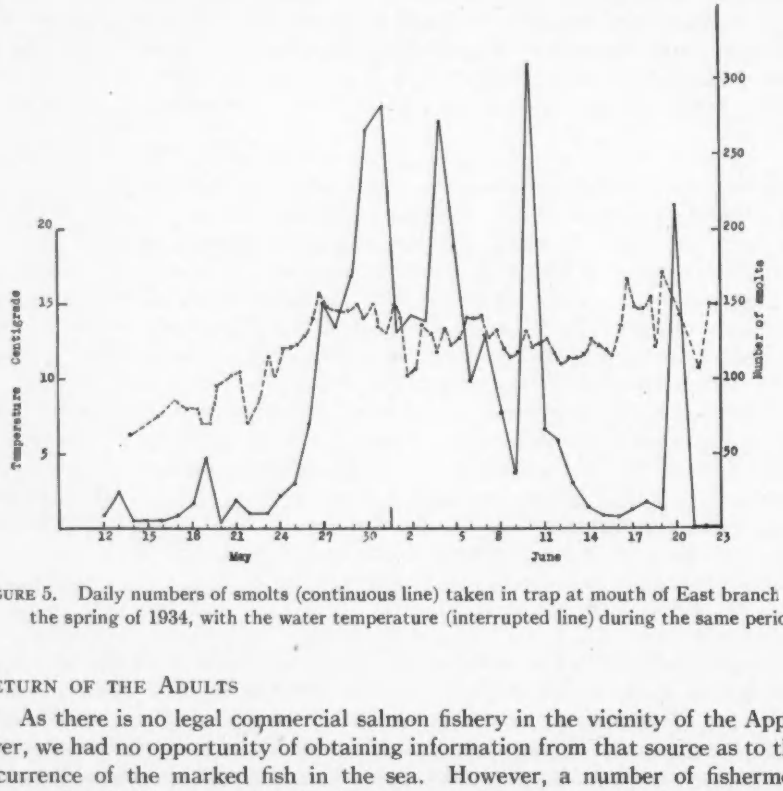

FIGURE 5. Daily numbers of smolts (continuous line) taken in trap at mouth of East branch in the spring of 1934, with the water temperature (interrupted line) during the same period.

RETURN OF THE ADULTS

As there is no legal commercial salmon fishery in the vicinity of the Apple river, we had no opportunity of obtaining information from that source as to the occurrence of the marked fish in the sea. However, a number of fishermen operate illegal gill nets at night in the estuary, and we requested these fishermen to look over their catches and report the presence of marked fish. In August 1935, the year following the marking of the smolts, we received word that fish with the adipose fin missing were common among their catches. We returned at once to Apple river and had a barrier and trap placed in each branch. At the same time (August 20) we examined several miles of the East branch and a number of pools on the South branch. The streams were low and visibility in the pools was good, but no salmon were seen. Local anglers and other persons who had been along the river previous to our arrival stated that no salmon had been seen in the fresh water.

Soon after placing the traps the run of salmon into the fresh water began. In a previous paper (White 1936b) this run has been described from the standpoint of the homing of the salmon. From August 30 to November 3, there were 92 marked grilse taken in the East branch trap and 6 in the South branch, all of which had been two-winter smolts. It is naturally uncertain how many had been taken by gill nets in the estuary.

In 1936 the gill netters reported taking the first large marked salmon with unmarked grilse in the estuary early in July. The traps were again installed and 5 marked virgin salmon were taken, 4 in the East branch and 1 in the South, all of these having been two-winter smolts, and with somewhat more than two years of sea growth, their lengths ranging from 75 to 80 cm. There was also a return of 20 of the marked fish which had spawned as grilse the previous year, 19 to the East branch and 1 to the South branch, their lengths ranging from 65 to 73 cm. Again it is uncertain how many were taken by nets in the estuary.

Straying

There is evidence to show that salmon at times enter rivers not their own, for example the eight marked fish of the East branch that were captured entering the South branch. Since no traps were operated on other streams in the region there is no means of knowing whether any of the marked fish entered any streams other than the two branches of Apple river. Salmon are, however, taken in the sea pretty much all around the upper end of the bay of Fundy.

In 1935 Mr. Forrest Watson, a salmon fisherman of Halls Harbour, N.S., on the south side of Minas channel, reported having seen around the end of July a grilse with the small dorsal fin missing. In 1936 at our request the Department of Fisheries sent a circular letter to salmon fishermen around the upper end of the bay of Fundy from Port George, N.S., to cape Enrage, N.B., asking them to report the occurrence of any salmon with the small back fin missing. One of 10 to 12 lb. (4½ to 5½ kg.) weight was reported as taken from a weir at Halls Harbour on June 26, corresponding in size with the marked two-sea-year virgin fish taken that year in Apple river. No marked fish were reported from any other locality, except a doubtful record for the head of Cobequid bay.

DISCUSSION

While it cannot be precisely stated for the salmon that furnished the Restigouche fry for the experiment, from what stream or streams they came, or what streams they would have entered and when, nevertheless they were from a region noted for its early-running fish, they were reportedly part of a catch of fish that was most abundant in June, the month when most of them were obtained, and they were, by inference, dominantly fish with three years of river life and returning after two or three full years in the sea. They thus formed a marked contrast with the Apple river salmon.

It was believed that the East branch of the Apple river in which the fry were to be planted was devoid of salmon, reports to that effect being confirmed by examination. Nevertheless, a few smolts were observed in it during the

spring of 1932, and 11 three-winter smolts that could not have been from the Restigouche fry, were taken in the trap operated in the spring of 1934. Also salmon ascended the East branch in the autumns of 1932, 1933 and 1934 and spawned, as shown by their offspring being in the stream the following season. This has been interpreted (White 1934, p. 41) as the effect of the introduction of young salmon into the stream. It is certain, however, that young salmon were extremely rare in the stream prior to the introduction of the Restigouche fry, none having been found in 1931, nor in 1932 except where the fry had been planted. There can be no question that the great bulk of the smolts marked on their descent of the East branch in 1934 were from the Restigouche fry.

The outcome of the experiment is, however, quite clear. The Restigouche fish remained only two years in Apple river instead of the three years characteristic of their native stream. Also they returned from the sea predominantly (95 per cent) as grilse instead of as two-sea-year and three-sea-year fish, the behaviour in their native region. Finally, while in the Restigouche river a large number of the fish return as early-run fish and ascend the river as such, those introduced into Apple river did not on return ascend as early-run fish, but were found both early and late in the estuary in common with the native stock. Water conditions in the river were probably not suitable for an early ascent, but later in the summer when there was sufficient water they did not ascend. Except in rate of growth in the river, for which obvious reasons have been given, the Restigouche salmon introduced into Apple river could not be distinguished by their behaviour from the indigenous salmon, and hence failed to show any evidence of a "Restigouche" inheritance. Although the failure is definite as to any racial distinction between Restigouche and Apple river salmon, the possibility of there being such distinctions in other places is not excluded. The significant point is that in this instance there has been a demonstration that environmental conditions, acting on the individual from the fry stage on, make the full observed difference in behaviour between Restigouche and Apple river salmon, almost as great a difference as is to be found in the salmon in Canadian waters.

The existence of races of *Salmo salar* is entirely problematical. Salmon living in lakes have been described as subspecies or varieties such as *Salmo salar sebago* of the lakes of Maine, but there has been no proof that the differences are more than the effect of the environment on the individual during its life. The average number of vertebrae has been used to characterize supposed races of herring and other fishes, but Mottley (1937) has shown how this is modified in *Salmo* by experimental conditions. Herring spawn at different times in the year and some have thought that races could be distinguished on this basis, but in the salmon there is one spawning time whether the fish run early or late, and this fails to provide a means of keeping the two groups distinct genetically. The facts that individual fish have been shown to enter different rivers in successive years and to move coastwise from south-western Nova Scotia to the north shore of the gulf of St. Lawrence (Huntsman 1931, p. 92), and from the bay of Fundy to Newfoundland and northern Labrador (Huntsman 1937b, p. 314) indicate the probability that intermixing of stock will tend to prevent the development of separate races in the various rivers. It should not, therefore, be considered

surprising that our experiment, made as crucial as possible, has so signally failed to show the inheritance of characters believed to be racial.

The elucidation of the factors responsible for the local differences in salmon behaviour is another matter. The fact that there may be such varied behaviour for the one river is an indication of the complexity of the matter. However, there are definite indications that the time spent by the young in the streams is inversely related to the suitability of the local temperature and food supply for rapid growth, and that significant differences may be found within comparatively short distances in one stream. Also the facts suggest that the number of years spent by salmon in the sea before first return, which largely determines the size, is, at least in part, dependent upon the ease of return (Huntsman 1936, p. 12). Temperature and food supply are also likely to be significant factors in the sea as they are in the early life in the river.

The season of return, which is of such great practical importance, is very far from presenting the clear picture envisaged in the simple idea of early-running and late-running fish. Occurrence of salmon in numbers near the surface or on the coast is not necessarily related to entrance into a river. The Apple river fish are on the coast and in the estuary from June on, but do not ascend the fresh water until late August or September. Big salmon appear early in May on the south shore of Minas channel, but there is no report of a run of fish of such size entering any river of the bay of Fundy, and they have been held to be "lost" salmon. The November-December run of 9 lb. (4 kg.) female Saint John salmon into the estuary in small part continues upstream from tidal water that autumn, but for the most part the fish remain for five or six months in the estuary, (where they may be taken through the ice during the winter), and ascend the river in the month of May, being known as the Serpentine run. When recaptures of salmon tagged just outside the mouth of the Margaree river and weekly angling catches in the river are compared with the data of river discharge, it is seen that entrance into the river depends upon the occurrence of satisfactory freshets, which may not come for several months after the salmon appear near the river mouth (Huntsman 1936 and 1937a). It may be confidently affirmed that knowledge of the hydrography of the river and of the adjacent sea will reveal the basis for varying local behaviour in the salmon.

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On Adequate Quantitative Sampling of the Pelagic Net Plankton of a Lake*

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ABSTRACT

Of the various kinds of error which arise in quantitative plankton investigations, those involved in the process of enumeration of a collection are ordinarily smallest in magnitude, though fractioning may introduce an extra sampling error, particularly when not done volumetrically.

More serious are the possibilities of error in the collection of the plankters from the lake. Traps are in general more accurate than nets, but the latter are more convenient, and when made of no. 10 silk are reliable quantitative collectors of the larger organisms. The more usual no. 20 silk is very variable in efficiency. Both nets and traps appear to suffer from the ability of some plankters to see and avoid them, by day. The sampling error of a collection, considered as representing the part of the lake near which it was taken, is such as to make a single collection, containing only a moderate number of individuals, of little value in determining abundance of a species.

On Cultus lake, collections taken at a single central station will indicate the average abundance of the pelagic plankton almost as precisely as would the same number of collections taken at various points throughout the whole pelagic region.

INTRODUCTION

Those who study lake plankton quantitatively have always found it difficult to estimate the accuracy of their results. Yet when only a limited time is available for an investigation, it is desirable to know what methods will give most accurate and representative data. Conversely, when a certain standard of accuracy is essential, it is important to know how it may be most easily obtained.

In sampling pelagic net plankton over a period of years, as a part of the sockeye salmon investigations on Cultus lake, British Columbia, experiments of various kinds have been made which have helped to answer the above questions. Many of the results obtained are of general applicability, and may be of assistance to those who face similar problems. Some of the data have already been published (Ricker 1933, 1937a), and brief summaries of them are included in the text below.

The difficulties and sources of error inherent in plankton work may be divided into three groups: (1) Difficulties of enumeration—i.e., in estimating the number of organisms in the collection of plankton as taken. (2) Difficulties in making collections—i.e., in ascertaining exactly the volume of the water from which the

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collection was filtered. (3) Difficulties of representation—i.e., in deciding to what extent the collections made are representative of the larger body of water from which they were taken.

ENUMERATION

Procedure

At Cultus lake, enumeration of the plankters in a collection was accomplished by transferring a fraction of it to a special cell under a binocular microscope. Collections were made up to a definite volume, 10, 20 or 50 cc., and ordinarily 5 cc. were removed in a special broad-mouthed suction pipette. (In the case of some collections made with a large net, a 1 cc. Stempel, or a 1 cc. suction-pipette was used for fractioning). The cell used had an area of 25 square centimetres and a capacity of 5 cubic centimetres; its glass bottom was ruled into twelve equal columns to aid in counting. The columns were also useful for further fractioning of a sample, when a dilution which was sufficient for the adult entomostracans proved too little for the smaller organisms; in such cases every other column or every third column was counted.

Counting

Errors in the actual counting of plankton organisms may occur, particularly when large numbers are being counted. Each enumerator must exercise care to avoid them, and make occasional recounts to check on himself. In the writer's experience, the probability of error of this kind is not sufficient to warrant complete duplication of counts.

Fractioning

When the collection is fractioned prior to counting, as described above, inaccurate volumetric fractioning is a possible source of error. Technique may be easily checked, however, by removing the whole of the given volume with the fractioning apparatus.

Sampling Errors

The count of a fraction of a collection is subject to a sampling error. It has been shown (Ricker 1937a, p. 75) that its probable extent may be estimated from the charts of fiducial limits of Clopper and Pearson (1934). If the number counted is fairly large (greater than about 30), the error to be expected is approximately represented by a standard deviation of $\pm\sqrt{Cq}$, where C is the number counted and q the size of the fraction which is *not* taken from the collection.

The above results obtain when the organisms are randomly mixed in the collection prior to sampling. If random mixture is not obtained, the sampling error to be expected will be greater than that indicated. This fact may be used to discover whether or not any given procedure does in fact provide a random sample of the collection. It has been found (Ricker, loc. cit., and from data in table I of Ricker 1933) that when fractioning is done volumetrically, using either the Stempel or the broad-mouthed suction pipettes, the fraction taken is a random sample of the organisms in the collection, or very close to it. On the other hand,

when fractioning was done by counting four only of twelve equal columns into which a collection was divided on a slide, the sampling error was significantly greater than expectation, in spite of efforts to distribute the organisms randomly over the slide. This last method, which was used for the smaller rotifers and algae, is therefore somewhat less reliable than the other, and the counts are subject to greater sampling error.

COLLECTION

Plankton Traps

A plankton trap, which encloses and brings to the surface a known volume of water from any desired depth, appears to be the most accurate type of collecting apparatus for macroplankton. On Cultus lake the 10-litre trap designed by Dr. C. Juday and constructed by Foerst Scientific Specialties, Chicago, has been used with excellent success. From the standpoint of accuracy, its only weakness appears to be that the more active plankters can see and avoid it, when it is used in the upper layers of water.

The trap suffers from two drawbacks, however. It is relatively unwieldy as compared with a net, and it can only with difficulty be used to obtain a representative average sample of the plankton of a deep column of water, such as is given by a single "total vertical" haul with a net. On the other hand, it is ideal for examining the vertical distribution of plankton.

Plankton Pumps

Pumps are useful, and almost essential, when large quantities of the smaller plankton organisms are needed, as for chemical analysis. To be effective they must be power-driven, hence require a large boat, and in general are the most unwieldy of the three types of apparatus. For collecting the larger or net plankters in large numbers a pump is not essential, though it may be very useful. These can be taken in nets, which, if carefully chosen and properly standardized, will give reasonably accurate results.

A Bunwell no. 1 wing pump, worked by hand, was used on Cultus lake to check the efficiency of plankton nets in 1932. It proved fairly well adapted to this purpose, but too tedious and unwieldy for making general collections.

Fine-meshed Plankton Nets

Four kinds of fine-meshed (no. 20 silk) plankton nets have been used on Cultus lake. Their specifications were as follows:

Net	Inside diameter of rings, cm. Upper	Inside diameter of rings, cm. Lower	Slant height of upper cone cm.	Straining cone Slant ht. cm.	Straining cone Area sq. cm.
(1) Medium	20	24	28	54	2450
(2) Small	10	16.5	40	39	1320
(3) Wisconsin-B.C.*	12	18	25	30	1090
(4) Rawson	30	45	55	75	5920

*This net is of the "Wisconsin" type described by Juday (1916, p. 569) and named by Reighard (1918, p. 74), but has a longer canvas cone at top. It is the same as the "Juday" net of Ricker (1933) and as the "Wisconsin" net of Rawson (1934). In the 1933 paper, the writer erroneously applied the name "Wisconsin" to Juday's larger closing net (1916, p. 573).

Their efficiency has been tested by comparison with the pump mentioned above, and the data have been presented in detail elsewhere (Ricker 1933). The more important results may be summarized as follows:

A net of no. 20 silk will shrink very considerably when first wetted; a new net should therefore be wetted and shrunk thoroughly before being used, to avoid greater changes in efficiency than are necessary.

The rate at which a mesh 20 net is hauled affects the abundance of its catch, the catch increasing as the rate increases, up to about 1 metre per second at least. (Tests were made in summer.)

A mesh 20 net, used to take several hauls in succession, decreases in efficiency throughout the first few. After five hauls have been made, the net is only two-thirds to one-half as efficient as in the beginning.

The efficiency of a mesh 20 net decreases with age. The extent of this decrease varied somewhat with the various nets used. An extreme case was that of a Wisconsin-B.C. net which after 10 months of use was only 7 per cent efficient at the time when a new net of the same design was 18 per cent efficient. Since the 1933 paper was published, evidence has been obtained that in extreme old age a net may again increase in efficiency.

Of these four sources of error, the first can easily be avoided. Also, the rate of haul can be—and at Cultus lake has been—kept constant at the standard rate of half a metre per second by checking the amount of line hauled for each ten seconds elapsed. But since the last two sources of error cannot readily be avoided, it is evident that large variations in efficiency are the rule rather than the exception with nets of no. 20 silk. In 1933, particularly, an attempt was made to keep a check upon changes in efficiency by frequent standardization, but the results were far from satisfactory—even old nets were very variable.

Coarse-meshed Plankton Nets

In searching for a more reliable sampler than no. 20 silk, larger meshes were obtained, and made into nets of the Wisconsin-B.C. pattern. The trade numbers of the meshes used, and the numbers of threads per centimetre in each, when the silk has been thoroughly shrunk, are given in the schedule below. The web has twisted threads running in one direction only, and the number of these differs slightly from that of the threads in the other direction.

Mesh no.	Twisted threads No. per cm.	Untwisted threads No. per cm.
OXX	15	16.5
3	23	24
6	29.5	32
10	44.5	48
20	77	81

On March 17, 1933, two vertical hauls through 40 metres of water at 0.5 metres per second were made with each of six nets, including an old mesh 20, a new mesh 20, and new nets of meshes 10, 6, 3, and OXX silk. The new nets had been previously wetted and shrunk; all carried buckets of no. 20 silk. At the same time two hauls were taken with the pump and strained through no. 20 silk

for the purpose of determining the factors of the nets, a net's factor being the reciprocal of its efficiency.

Table I summarizes the data obtained in these tests. Since only two of each kind of haul were made, the figures have no great reliability. This is evident from the estimated sampling errors, which, calculated on the basis of random distribution of the plankters, are themselves minimal (Ricker 1937a). It is, however, clear that efficiency increases rapidly with increase in size of mesh, being 0.2 for a new no. 20 net, while those for larger meshes cannot with certainty be distinguished from 1.0.

TABLE I. Kinds and numbers of organisms caught in two total vertical (40 m.) hauls, by the pump and by nets of various meshes, March 17, 1933. Figures in the first column represent the number of organisms taken, divided by the volume of water pumped up and strained (68.2 litres in two hauls). Other *unbracketed* figures represent the number of organisms caught by the net in question, divided by the volume of the column of water which the net would strain if it offered no resistance to the passage of water (906 litres in two hauls). *Bracketed* figures for mesh 20 nets represent the number of organisms caught, divided by the volume of water actually strained by the net. They are calculated by multiplying the corresponding unbracketed figure by the average net factor. For nets other than mesh 20 the factor is close to unity, and this calculation has been omitted.

	Pump	Mesh OXX	Mesh 3	Mesh 6	Mesh 10	Mesh 20 (new)	Mesh 20 (old)
Straining area, cm^2.		1180	1130	1150	1140	1070	1020
Melosira (mm. filament)	165	26	21	91	2620	1420 (5800)	174 (2660)
Asterionella (colonies)	1047	32	18	70	415	413 (1690)	152 (2330)
Conochilus	2.29	0.03	0.82	3.03	2.16	0.73 (3.0)	0.26 (4.0)
Asplanchna	1.67	0.03	0.81	2.22	1.45	0.43 (1.7)	0.12 (1.9)
Synchaeta	2.23	0.04	0.03	0.00	1.39	0.70 (2.9)	0.17 (2.6)
Polyarthra	1.82	0.07	0.06	0.19	0.19	0.88 (3.6)	0.26 (4.0)
K. quadrata	0.29	0.01	0.01	0.03	0.16	0.13 (0.5)	0.05 (0.8)
K. cochlearis	1.29	0.04	0.04	0.03	0.63	0.88 (3.6)	0.34 (5.2)
Notholca	4.34	0.11	0.15	0.20	4.78	1.18 (4.8)	0.53 (8.2)
Nauplii	4.63	0.11	0.07	0.14	1.01	1.13 (4.6)	0.36 (5.5)
Cyclops	12.35	0.20	0.39	6.69	10.10	2.72 (11.1)	0.68 (10.4)
Bosmina	1.17	0.01	1.05	1.20	1.72	0.31 (1.3)	0.10 (1.6)
Daphnia	0.13	0.05	0.11	0.22	0.14	0.03 (0.1)	0.01 (0.2)
Average net factor				0.82	1.08	4.09	15.3
Sampling error, as standard deviation				0.091	0.073	0.27	0.63

The reaction of the various organisms may be discussed by groups:

DIATOMS

Melosira is retained almost completely by no. 20 and about half of it remains on no. 10 silk, but it escapes larger meshes. It is, however, small enough to slip through even no. 20 end on, and when, as in the pump hauls, the water is much agitated against the silk, most of the filaments apparently do so. *Asterionella* is probably completely retained by no. 20 but about ¾ of the colonies escape the no. 10.

ROTIFERS

The small rotifers *Keratella cochlearis*, *K. aculeata* and *Polyarthra* readily pass through all the silks used except the no. 20. It is probable that some may even escape this mesh, because extra agitation appears to reduce the number retained. The larger *Synchaeta* are all retained by no. 20, and about half are caught by no. 10. Long-spined *Notholca* cannot pass either of these two, but for the most part escape through no. 6. The large *Asplanchna* and gelatinous colonies of *Conochilus* are apparently all strained out by meshes as large as no. 6.

TABLE II. Comparison of the counts from total vertical plankton hauls with a mesh 20 silk Wisconsin-B.C. net, taken at points scattered over a large part of the open water of the lake (July), and at a central point (September). Counts of Entomostraca are of the total collection, of *Ceratium* only one-tenth of it.

	Epischura		Cyclops		Daphnia		Ceratium
Haul number	July	Sept.	July	Sept.	July	Sept.	Sept.
1	21	12	74	487	63	37	169
2	14	5	122	452	54	48	149
3	6	6	92	417	91	37	143
4	5	8	81	460	83	46	159
5	6	8	104	546	104	38	152
6	11	8	92	514	79	38	152
7	8	7	90	392	71	50	160
8	-	4	-	483	-	33	140
9	-	4	-	530	-	35	149
10	-	10	-	499	-	50	192
Totals	71	72	655	4780	545	412	1565
Mean ($\bar{x}$)	10.1	7.2	93.6	478.0	77.9	41.2	156.5
Sum of squares of deviations	198.8	59.6	1477.0	21288.0	1721.0	386.0	2042.0
Variance (s^2)	33.3	6.62	246.0	2365.0	287.0	42.9	227.0
Variance/Mean (s^2/x)	3.30	0.92	2.63	4.95	3.69	1.04	1.45
χ^2	19.7	8.3	15.8	44.6	22.1	9.4	13.1
Probability (P)	0.003	0.50	0.015	0.000	0.001	0.40	0.15

ENTOMOSTRACANS

Very few copepod nauplii appear to pass through no. 20 gauze, but only a quarter of them are stopped by no. 10. Adult *Cyclops* are most common in the

no. 10 net, and good numbers of the larger ones appear in the no. 6. *Bosmina* too are most abundant in no. 10, but are fairly common even in the no. 3. The most efficient collector of the small specimens of *Daphnia* found in the lake at this time appears to be no. 6 silk; no. 3 allows about half of them to escape.

From this analysis it appears that a net of no. 10 silk can be used as a quantitative collector of adult entomostracans, filamentous algae, and the larger rotifers, but not of copepod nauplii, colonial algae, the smaller rotifers, and probably not of protozoans (*Ceratium*, *Dinobryon*).

As the Entomostraca were of most immediate interest at Cultus lake, a no. 10 mesh net has been used from December, 1933, to the present for determining their average abundance in a total vertical haul. For these organisms, it has proved greatly superior to the no. 20 nets, because (1) there appears to be no change in its efficiency when a series of as many as 20 hauls are taken in quick succession (table III below), and (2) over a period of service of a year and a half, no *significant* change in efficiency occurred, although toward the close of that period the trend appears to be toward a slight reduction.

Factors obtained for this net are as follows:

1933: March 17, 1.08.

1934: June 4, 1.08; June 16, 1.46; July 4, 1.14; July 17, 0.96; August 1, 1.20; August 16, 1.13; August 31, 1.03; September 14, 1.09; October 1, 1.00; October 17, 0.93; November 2, 1.23; November 18, 0.92; December 4, 1.21; December 30, 0.97.

1935: February 1, 0.87; February 15, 1.47; March 1, 1.24; March 20, 0.91; April 5, 1.72; April 19, 1.25; May 2, 1.17; May 20, 1.11; June 5, 1.41.

Mean of	1934 factors:	1.105;	standard deviation =	0.156;	st. error of mean		0.043.
"	1935 "	1.212;	" "	0.268;	"	"	0.085.
"	all "	1.151;	" "	0.211;	"	"	0.044.

The 1933 standardization was accomplished by means of the pump hauls described above, the others by comparison with the average of 9 trap catches taken 5 metres apart on the same day, giving the top and bottom ones half of the weight of the remainder. Counts of Entomostraca only were used in calculations. The factor for a new net of this type is apparently close to 1.10.

Relation of "Stage" Hauls to Total Vertical Hauls

Early sampling at Cultus lake was by means of "stage" hauls of the fine-meshed net, usually in 5-metre stages from 0 to 10 metres, and in 10-metre stages from 10 to 40 metres. In 1932 and 1933 both stage hauls and total vertical hauls were made. In theory, the sum of the plankters in the stage hauls should equal the number in the total vertical haul on any given date, but in practice many planktologists have found that this is not the case, the sum of the stage hauls being almost always less than the total vertical. This has resulted in a theory that some of the plankters of a stage haul are "spilled" from the net when it closes.

Whatever the reason, the effect is very noticeable in the Cultus lake data, and is such as to make the stage hauls of little value for determining vertical distribution of plankton organisms. It was desired, however, to make use of stage hauls taken prior to 1932 to determine, as well as possible, the average vertical

density of the plankton at that time. To this end the total vertical and stage hauls were compared over the two years 1932 and 1933, as shown in figure 1 for *Cylcops*. The ratio of the two has a distinct seasonal trend, being least in winter and greatest from July to September. It was also greater in 1933 than in 1932, although in the earlier year there was one stage less than in the later, the bottom 20 metres having been taken together. The solid line in figure 1 represents a freehand estimate of the average ratio of total-vertical to stage hauls, and was used in calculating average abundance when the former were not available.

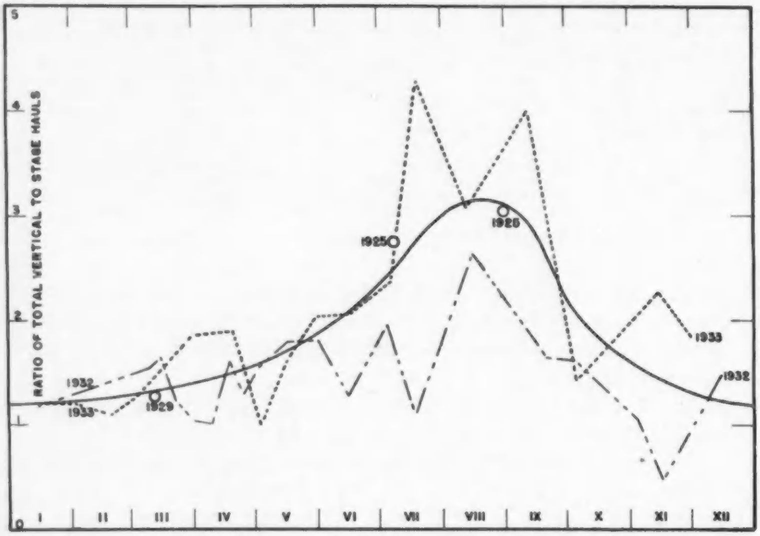

FIGURE 1. Seasonal variation in the ratio of the number of *Cyclops* caught in a total-vertical haul with no. 20 net, to the number caught in stage hauls. Data are for 1932 (fine-dotted line) and 1933 (coarse-dotted line), with two determinations for 1925 and one for 1929. The solid line is a free-hand average for all years.

A similar calculation was made for the relatively non-motile organism *Notholca* with results very similar to those for *Cyclops*. It appears, therefore, that activity of the plankters is not a factor responsible for this phenomenon.

Ability of Plankters to Avoid Capture

Southern and Gardiner (1926), among other limnologists, have observed that their nets caught fewer of the large entomostracans by day than during the hours of darkness. They favoured the view that this is because the animals are able to see the net and avoid it, since the phenomenon is most marked in the case of organisms whose preferred habitat is in the more brightly illuminated surface waters. The differences between the day and night catches in Lough Derg were of considerable magnitude in the case of large *Daphnia longispina*—a ratio of 2.4 being recorded in one instance. Other students have found even greater discrepancies.

Evidence that the same condition may obtain in Cultus lake may be had from table III below. The series of "scattered" hauls was made from 10.30 a.m. to 3.25 p.m.—the brightest part of a cloudy day; the hauls at the buoy were taken from 8.00 to 10.00 p.m., or dusk to dark. The mean counts of the buoy series may be compared either with the mean of the scattered series, or with that haul of the scattered series (no. 20) which was taken at the buoy. The single haul shows no great deviation from the dusk series, except in the cases of *Daphnia* and *Cylcops*, where it is less, there being about 1 chance in 20 in each case that it is accidentally so. The close agreement of the two means suggests that it *is* accident in the case of *Cyclops*, but on the other hand the very significant difference between the two *Daphnia* means indicates that there is a greater catch of these organisms in the buoy series than in the scattered series, the ratio being of the order of 71.4/42.6 or 1.7.

TABLE III. Comparison of total vertical plankton hauls with no. 10 silk Wisconsin-B.C. net, taken at a central point (buoy) July 4, 1935, and over the whole of the open water of the lake (scattered) July 5, 1935. Counts represent 1/25 of the total collection. (To change figures tabulated to read as number of organisms *per litre*, multiply by 0.0607.)

Haul	Epischura		Cyclops		Nauplii		Daphnia		Bosmina		Notholca	
number	Buoy	Scattered	Buoy	Scattered	Buoy	Scattered	Buoy	Scattered	Buoy	Scattered	Buoy	Scattered
1	1	7	128	131	51	37	53	41	2	2	16	9
2	6	7	107	121	66	50	63	39	0	1	14	12
3	6	3	122	157	56	52	61	50	0	1	14	16
4	5	9	133	138	62	49	95	44	0	3	11	10
5	7	9	119	134	66	61	71	56	0	1	12	10
6	11	6	127	85	60	52	97	60	4	2	10	19
7	8	2	123	119	53	48	88	36	0	3	17	23
8	2	4	127	131	58	66	85	54	0	4	11	28
9	8	5	121	153	62	41	78	20	0	5	16	24
10	3	3	126	122	54	46	52	29	1	1	13	10
11	3	9	120	128	59	62	72	41	2	1	13	16
12	3	8	109	111	56	45	62	36	3	2	24	20
13	5	8	110	129	52	50	65	47	0	4	14	14
14	3	1	127	-	60	-	71	40	3	1	8	-
15	6	5	125	-	42	-	75	48	1	2	8	-
16	3	6	120	-	64	-	74	38	1	3	8	-
17	6	6	134	105	59	65	65	44	2	1	12	7
18	6	1	115	-	60	-	74	38	1	3	21	-
19	6	8	126	112	60	46	64	41	1	1	12	13
20	3	4	125	107	65	58	64	47	2	0	19	14
21	-	6	-	126	-	37	-	47	-	1	-	17
Total	101	117	2444	2109	1165	866	1429	896	23	42	273	282
Mean ($\bar{x}$)	5.05	5.57	122.2	124.0	58.2	51.0	71.4	42.6	1.15	2.0	13.65	15.40
Sum of squares	113.0	132.0	1031.0	4394.0	652.0	1275.0	2738.0	1608.0	28.7	34.0	345.0	547.0
Variance (s^2)	5.95	6.57	54.3	274.0	34.3	79.7	144.0	80.4	1.51	1.70	18.14	34.2
$s^2/\bar{x}$	1.18	1.18	0.43	2.21	0.59	1.56	2.02	1.89	1.31	0.85	1.33	2.22
χ^2	22.4	23.7	8.4	35.4	11.2	25.0	38.4	37.8	25.0	17.0	25.3	35.5
Probability (P)	0.26	0.25	0.98	0.005	0.91	0.050	0.005	0.01	0.16	0.65	0.15	0.01

It is probable that this difference is owing to the difference in illumination, but singularly enough the *Epischura*, which inhabit the epilimnion principally, and are hence in a much better position to avoid the net than are most *Daphnia*, show no sign of any such reaction. The alternative explanation would involve an actual change in abundance of *Daphnia*, of the magnitude shown in a day's time. Unfortunately no series of day and night hauls at the buoy is available to

check this possibility, but it seems inconsistent with the picture of slow seasonal changes obtained from semi-monthly samples taken there.

No direct experiments were made to ascertain whether or not *Daphnia* will avoid the plankton trap, but indirect evidence shows that their reaction to it must be very similar to their reaction to the nets. When counts of Entomostraca from a series of trap catches were compared with those from a net haul for the purpose of finding the efficiency of the latter (see above), it was found that the ratio of trap to net catches was practically the same for *Daphnia*, *Cyclops* and *Epischura*, at all times of year. Hence if some *Daphnia* avoided the net, they must have avoided the trap as well, and in about the same degree.

The obvious way to eliminate the errors due to any possible avoidance reaction would be to make collections at twilight or after dark. This procedure has not been followed at Cultus lake, because of its inconvenience, and because its value was not suggested until late in the investigation. The collections, as taken during daytime, are comparable among themselves, but in using results calculated from them it is necessary to remember that summer catches of *Daphnia* may be considerably too small.

Sampling Error

A plankton collection as brought in from the lake may be considered a sample of the plankton of the water of the immediate vicinity from which it was taken. In so far as it is regarded as such a sample, it is liable to sampling error. It has been shown (Ricker 1937a, p. 78) that if the plankton in the region represented is randomly distributed, the probable limits of error of the number of organisms in the collection can be found in a table of fiducial limits for Poisson distributions, as given in Foerster 1936, Ricker 1937a and 1937b. If the number counted in such a collection is greater than about 50, the count will be subject to a standard deviation approximately equal to its own square root. It was further shown (Ricker 1937a) that the horizontal distribution of five pelagic plankton organisms, at a point in Cultus lake, was of this random type or nearly so, while *Daphnia* showed a small departure from random distribution.

Great care should be taken, however, in extending these results to other bodies of water, and limits of error calculated as above should always be considered minimal, unless proved otherwise. A simple calculation will show that, even on the basis of this minimal error, any population estimate from, for example, a single count of up to 200, can never achieve second figure accuracy. This fact makes frequent sampling, or replication of hauls, an essential part of any programme for accurately estimating a lake's population. In the latter case replicated hauls can be averaged; in the former, a running average will help to smooth out the chance fluctuations.

The process of fractioning a collection, considered as the taking of a sample of a sample, does not introduce any additional source of error into a count of a given size, as long as it is done in a technically satisfactory manner. If, however, it is not done in a wholly random fashion (as with the slide-fractioning mentioned above) it does produce an added error.

REPRESENTATION

Horizontal Distribution of Net Plankton

Considering a lake in its entirety, it cannot be expected, and it is never found, that its plankton is uniformly distributed horizontally, either per unit of volume or per unit of area. In general, the shallow littoral region will possess a fauna which is not only quantitatively different from that of the open water, but contains many species which the latter lacks. When a lake has large shallow bays, which contrast with the deep pelagic region, this littoral-type plankton may occupy quite a large area of the lake (cf. MacKay 1924).

It has been found, however, that in some lakes there exist differences in the quantity of plankton occupying different parts of the open-water or pelagic region, where the water ranges from moderately deep up to the maximum. Southern and Gardiner (1926) observed large differences between different stations on Lough Derg, which perhaps could be expected from its irregular depth contours and the effect of the large volume of water entering from the river Shannon. However, Ruttner (1930), working on the small, deep and rather uniform lower lake at Lunz, found that "kaum messbare Unterschiede der Aussenbedingungen können die Lebensfunktionen bald des einen, bald des anderen Plankton- organismus in merklicher Weise beeinflussen und durch Vermehrung oder auch durch Eigenbewegung eine relative Zunahme bzw. Abnahme einzelner Arten in verschiedenen Teilen des Gewässers bedingen." His data show several striking examples of non-uniform distribution between different points in the pelagic region of the lake, but he believes that these do not necessarily disprove uniform distribution per hectare, or even per are, of its surface. Naber (1933) found that the mean number and variability of distribution of various plankters in eight hauls from different stations in the pelagic region of the great lake of Plön did not significantly exceed their mean and variability in six hauls from a single station.

Experiments at Cultus lake

The large area of open water and the uniform depth of the pelagic region of Cultus lake (cf. Ricker 1937c) make it *a priori* probable that, as in the Plöner See, there will not be great variation in kind or amount of plankton from point to point. But the importance of the question from the standpoint of sampling technique makes it essential to have definite information in this regard. If, for example, there were twice as many *Daphnia* in the middle of the lake as at either end, the economy of effort obtained by making one station representative of the whole could be achieved only with great loss of accuracy.

Accordingly several experiments have been made. On July 21, 1933, seven total vertical hauls were taken with a mesh 20 Wisconsin-B.C. net at points scattered over the pelagic region of the lake, the net being dried between each haul in order to avoid the decline in efficiency characteristic of the fine mesh. On September 2, 1932, ten similar hauls with dry net were taken at the central buoy. The two series of data are given in table II, *Epischura*, *Cyclops* and *Daphnia* having been enumerated in both cases, and *Ceratium* in the latter case only.

A more careful study of variability of distributions was made in 1935. On July 4 there were taken, between 10.30 a.m. and 3.25 p.m., 21 hauls with the mesh no. 10 Wisconsin-B.C. net, at points widely and rather uniformly scattered over the deep water of the lake. On July 5, with the same net 20 hauls were taken at the buoy, between 8.00 and 10.00 p.m. Counts of 1/25 of each haul are set forth in table III.

In both tables the mean, sum of squares of deviations from the mean, and variance of each series are recorded. The quotient Variance/Mean, which may be called the "relative variance" of the series, gives an index of the type of distribution of the plankter in question. Random distribution horizontally is indicated by a relative variance of unity. A larger value suggests that the plankters are somewhat bunched, a smaller value would suggest that the plankters were spaced more evenly than randomly in cases where the whole rather than only a fraction of each collection was counted. To test whether or not observed differences from unity are significant, the χ^2 test is used, with $\chi^2 = S(x-\bar{x})^2/\bar{x}$. Probabilities were found from a table of χ^2, and are tabulated. Values from 0.95 to 0.05 offer no indication that distribution is other than random. Decreasing beyond 0.05 they suggest with increasing emphasis that distribution is bunched.

Of the ten series of counts from collections taken at a point, six show no departure from a random toward a bunched distribution. Four appear to be significantly bunched (*Cyclops* in table II; *Daphnia*, *Bosmina* and *Notholca* in table III). (The *Cyclops* counts of table III are peculiar in indicating that chances are 1 in 50 that they constitute a series with less than normal variance. However, since the fractioning process introduces a variance equal to 24/25 of that characteristic of random distribution in the lake, cf. above, it can confidently be said that the suggestion of "spacing" is accidental.) Of the nine series of counts from "scattered" samples, most indicate clearly that the distribution was somewhat more variable than random over the lake, exceptions being *Epischura* and *Bosmina* in table III. It can be concluded that the horizontal distribution of plankters over the whole pelagic region of Cultus lake is ordinarily more uneven than random, though not greatly so.

More important, however, is the question of whether the *mean* number of organisms taken at the buoy differs significantly from the average for the whole lake. Table III gives this information, for the two series of hauls were taken on successive days and the lake's population could have changed little in so short a time. Differences between the pairs of "buoy" and "scattered" means are for the most part quite small. Tested by means of the "t" distribution of Fisher (1932), only one stands out as significant: there are fewer *Daphnia* in the scattered series. However, as explained above, this is in all likelihood because the two series were taken at different times of day. Other tests can be made by dividing up and comparing the hauls of the "scattered" series according to their position on the lake's surface, *e.g.*, northern with southern, peripheral with central, etc. Such comparisons are not tabulated, but none showed any evidence of a concentration in any particular situation.

It can, then, fairly be said that the method of choosing a central station to

represent the whole pelagic region of Cultus lake is in general quite satisfactory. Usually, however, the sampling error of counts made from collections there will be somewhat greater than that calculable on the basis of random distribution.

SUMMARY

The difficulties in obtaining an accurate estimate of the abundance of the various organisms in the pelagic net plankton of a lake are of three kinds, (1) those involving the actual counting, (2) those involving the method of collecting the plankton, and (3) those concerning the validity of considering the collections made as representative of the whole pelagic region of the lake.

(1) Counting is itself an operation involving little chance of error, and this of purely accidental kind. Commonly only a fraction of a collection is counted, and in this case the count is subject to a sampling error, whose magnitude may readily be calculated. As the collection itself is subject to a sampling error of as great or greater magnitude, the error of fractioning does not introduce additional uncertainty into a count of given size, as long as the technique used is accurate, i.e. purely random. It was found that volumetric methods of fractioning approach this ideal, but that fractioning on a slide is not as satisfactory.

(2) In making plankton collections, the great desideratum is that the exact volume of water which is filtered should be known. The plankton trap provides this information most directly, but for some purposes is less convenient to use than a net. The latter, when made of no. 20 silk, strains a volume of water which varies with a number of conditions, and hence is of little use quantitatively unless frequently standardized. A net of no. 10 silk, however, appears to be unusually constant in efficiency, even over long periods, and makes an excellent sampling apparatus for adult Entomostraca and the larger rotifers.

Owing to the possibility of active plankters avoiding the apparatus, collections taken for the purpose of estimating their absolute abundance should be made after dark. On Cultus lake *Daphnia* appears able to avoid both net and trap to some extent, in summer at least.

When vertical hauls are made by stages with the no. 20 net, the numbers of both active and inactive plankters which they catch are almost always less than those taken in a single haul through the same total distance. On Cultus lake the ratio of total-vertical to stage hauls was found to change seasonally, from 1.2 in winter to about 3.1 in August, but with great individual variation.

When considered as representing any part of a lake greater than the actual volume from which it was strained, a plankton collection is subject to a sampling error. The expected magnitude of this error can readily be calculated, on the assumption that plankters are randomly distributed in the region of the lake which the collection represents; any departure from random toward bunched distribution makes errors so calculated too low, so they must usually be regarded as minimal. Their probable magnitude, even when fairly large counts are made, is such that a single collection will represent the plankton at a station on the lake only with a considerable degree of uncertainty.

(3) Tests made on Cultus lake demonstrate a horizontal distribution of

plankters, as taken in total-vertical net hauls over the whole pelagic region, which does not depart very greatly from a random distribution. Further, there appeared to be no tendency toward concentration of any of the species investigated (4 entomostracans, 2 rotifers) in any local region of the lake. It appears, therefore, that collections made at a single central station on the lake would adequately represent the whole pelagic region, provided they were made sufficiently frequently to bring the sampling error within reasonable bounds. Tests made by others suggest that this condition may be fairly general in deep lakes of small to medium size and uniform bottom configuration, but are insufficient to establish a general rule.

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Seasonal and Annual Variations in Quantity of Pelagic Net Plankton, Cultus Lake, British Columbia*

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ABSTRACT

Intermittently over a 13 year period the net plankton of Cultus lake has been sampled, at a central representative station. Net plankton was not particularly rich in common species. Of the entomostracans important as fish food, only four occurred. Seasonal distribution of the various species is of two principal kinds: unimodal, with one peak of abundance in late spring or summer—various green algae, Protozoa, Rotatoria, Cladocera and *Epischura;* and bimodal with peaks in early spring and in autumn—diatoms, mostly Rotatoria and *Cyclops*. Among bimodal species the spring maximum is ordinarily the greater. Marked differences in abundance of a plankter occur from year to year and affect both the maximal numbers attained and duration of time of proliferation. The available data do not show these annual changes to be cyclic and no definite correlation with environmental conditions has been made. The total nitrogen content of net plankton varied from 2 to 10 mg. per l. in 1932, the dry weight from 30 to 210 mg.

INTRODUCTION

The plankton collections upon which this paper is based were taken in the course of a limnological study of Cultus lake, carried on in conjunction with the investigation of the life history and propagation of the sockeye salmon (*Oncorhynchus nerka* Walbaum) outlined by Foerster (1929).

In the first paper of this series (Ricker 1937b) it was shown that much the greater part of the lake bottom is profundal, lying below the depth of twenty metres, while the shallower sublittoral and littoral areas are of comparatively limited extent. Since larger plant growth does not extend into the profundal region, and such plants are only patchily developed even in the shallower areas, the great bulk of the lake's primary foodstuffs must be elaborated by the plants included in the pelagic plankton of the open water. Because of its predominant importance in the lake's metabolism, this pelagic plankton alone has been studied in the Cultus lake investigation, and littoral plankton ignored. This was done the more readily as the principal object of the plankton study has been to determine its ecological relationship to the young sockeye salmon inhabiting the lake, and observations indicate that these fish avoid the littoral region almost completely.

The plankton study has been further restricted to the larger or "net" organisms, i.e., those which are caught, at least partly, by silk nets no smaller

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than mesh no. 20. Such eclecticism can be justified only because of the press of other work, and because of unfamiliarity with the easiest method of enumerating the smaller plankters, i.e., with the aid of the Utermöhl or "upsidedown" microscope.

The first study of the lake's plankton was carried out by Foerster (1925), who made collections at several stations throughout the summer of 1923. These provide a picture of the relative, and to some extent the absolute, abundance of the various organisms in that year. In 1924, 1925, and 1927 to 1929 Dr. Foerster took monthly samples at a single representative station in the open lake, which have lately been examined by the writer. Plankton investigations were resumed at the beginning of 1932 and have been continued to the summer of 1935. Thus there are data extending, intermittently, over a period of 13 years, bearing on the plankton plants and animals of the lake.

METHODS

Apparatus

The following kinds of apparatus have been used from time to time to sample the plankton:

(1) Medium net of no. 20 bolting silk, used 1923 to 1925.
(2) Small net of no. 20 silk, used 1927 to 1929.
(3) "Wisconsin-B.C." net of no. 20 silk, used in 1932 and 1933.
(4) "Rawson" net of no. 20 silk, designed by Dr. D. S. Rawson for the purpose of taking plankton for micro-Kjeldahl nitrogen analyses; used in 1932 and 1933.
(5) "Wisconsin-B.C." net of no. 10 silk, used from late 1933 to 1935.
(6) Plankton trap of 10 litres capacity.
(7) Wing pump, used to standardize nets in 1932 and 1933.

A full description of the nets and other apparatus mentioned above has been given in earlier papers (Ricker 1933, 1937c, 1938a).

Collections Made

Collections have been made at a central station on the lake as follows:

1923: 6 series of stage catches, July to October.
1924: 1 series in January; semi-monthly from September to December.
1925: mostly semi-monthly series, January to October.
1927: 3 series, September to December.
1928: 9 series, February to November.
1929: 5 series, February to June.

All of the above were series of stage hauls, usually taken at intervals of 10 metres in the lower part of the lake and 5 metres near the surface. Those from 1923 to 1925 were with the medium net, from 1927 to 1929 with the small net.

From 1932 more comprehensive collections were made as follows:

1932: total vertical hauls with Wisconsin-B.C. net and with Rawson net, for the most part semi-monthly, but four times a month in March and April, and once only in January and October.

1933: total vertical hauls with Wisconsin-B.C. and Rawson nets, semi-monthly except January, August, September and December, in each of which only one series was taken.

1934: total vertical hauls with a coarse-meshed Wisconsin-B.C. net (no. 10 mesh) semi-monthly from January to May, four times a month thereafter. Stage catches with the trap at five-metre intervals from surface to bottom (0 to 40 metres) semi-monthly from June onward.

1935: as in the latter part of 1934.

All collections were preserved in formalin, of strength varying from 5 per cent to 20 per cent.

PROCEDURE

In an earlier paper (Ricker 1938a) the methods of enumerating the plankton have been described in detail, and their accuracy discussed. The relative values of the various methods of taking collections were also examined. The mesh no. 20 nets were found to be quite variable in efficiency, as shown by the following determinations of their "factors" (reciprocals of efficiencies) in total vertical hauls of 40 metres in comparison with pump hauls:

Net number	W1 (used from Jan. 1932)	W2 (new)	W3 (new)	R1 (used 12 months)	R2 (new)
1932					
July 22-23 . . .	(10)				
Sept. 1-2	11.8				
Oct. 4	14.4	5.6			
1933					
Feb. 2	11.9	5.4		8.1	4.0
March 17	15.3		4.1		
Oct. 12	5.9				
Dec. 10	8.3				

W—Wisconsin-B.C. net. R—Rawson net.

Statistical sampling errors of the above have not been tabulated, but are quite large, so that the fluctuations noted under W1, which was the Wisconsin-B.C. net used up to the end of 1933, are not all significant. Nevertheless, the increase in efficiency of this net after many months of use is real, and rather puzzling. It may be that in old age a net's fibres become worn thin, enlarging the apertures again, and hence increasing the efficiency by allowing a greater volume of water to pass through. In calculating population densities for this paper, a free-hand graph along the points above was used—in which procedure there are possibilities of large errors.

Prior to 1932 hauls at the central station were taken in stages of 5 to 10 metres length. It was shown (Ricker 1938a) that the sum of such hauls rarely equals a single total-vertical haul, and that the relation between the two varies seasonally, the single haul taking relatively more plankters in summer than in

winter. The average relationship between the two, as presented in a chart in the earlier paper, was used to convert the early stage hauls into terms of total vertical hauls. At the same time the marked variability in this relationship prevents any accuracy attaching to samples taken in this manner. Summer average abundance so estimated will probably be subject to an accidental error of from half to twice the nominal value, at least. In addition, it was impossible to ascertain the efficiency of the nets used over this period, so they were assigned a factor of 5 throughout—which further increases the unreliability of their data. Nevertheless, these data are of some value, as the seasonal fluctuations in abundance of plankters are so great that trends show up quite clearly in many cases.

In 1934 and 1935 the abundance of the larger plankters could be more reliably estimated from the hauls with no. 10 mesh net (factor taken as 1.1 throughout), and from June 1934 to June 1935 the trap catches provide a relatively good index of the average abundance of all species. In these cases most of the technical errors have been eliminated, what variability remains being largely the result of unavoidable statistical sampling error (Ricker 1937a). An important exception however occurs in the case of *Daphnia*, which, probably owing to its ability to avoid the collecting apparatus, appears to be taken less abundantly in daytime collections of summer than its real numbers would warrant (Ricker 1938a).

It was shown (*ibid.*) that the central station was a satisfactory place for collecting the pelagic plankton of the lake, in the sense that a given number of collections provide results of nearly as great an accuracy when taken all at this station as when taken at points scattered over the entire pelagic region.

RESULTS

List of Genera and Species

Collections of plankton from the central station on Cultus lake reveal a considerable variety of species. Samples taken February 3, April 1, August 12, October 5, and December 1, 1933, also August 1, 1934, have been examined by Dr. E. H. Ahlstrom of the U.S. Bureau of Fisheries, who determined the algae, protozoans and rotifers listed below:

Diatoms: *Asterionella formosa*, *Amphora* sp., *Gyrosigma* sp., *Synedra ulna*, *Synedra cycloporum* (on copepods), *Fragilaria capucina*, *Amphiprora ornata*, *Campylodiscus hibernicus*, *Melosira varians*, *Melosira islandica helvetica* (the common vernal species), *Melosira* sp., *Rhopaladia gibba*, *Cyclotella* spp.

Green algae: *Ankistrodesmus falcatus*, *Sphaerocystis schroederi*, *Nephrocytium agardhianum*, *Oocystis solitaria*, *Oocystis lacustris*, *Crucigenia rectangularis*, *Cosmarium laene*, *Staurastrum* sp.

Blue-green algae: *Coelosphaerium kuetzingianum*.

Heterokontae: *Botryococcus braunii*.

Protozoa: *Dinobryon divergens*, *Ceratium hirundinella*.

Rotatoria: *Asplanchna priodonta, Conochiloides natans, Conochilus hippocrepis, Conochilus unicornis, Collotheca* (probably *mutabilis*), *Keratella cochlearis, Keratella quadrata, Polyarthra euryptera, Polyarthra trigla, Ploesoma hudsoni, Notholca longispina, Synchaeta pectinata.*

Collections of other years would probably add considerably to the algae of the list; the writer has observed *Mougeotia, Anabaena* and *Microcystis*. Foerster (1925) records a number of other genera, taken from inshore and open water habitats.

Only five arthropods appear in the plankton of the open water, of which four were entomostracans, listed by Foerster thus:

Copepoda: *Epischura nevadensis, Cyclops bicuspidatus.*
Cladocera: *Daphnia pulex, Bosmina obtusirostris.*

The fifth arthropod is tentatively identified as a nymphal water-mite (Hydracarina): it is of occasional occurrence in spring.

Fluctuations in Abundance

A long table has been prepared giving the numbers of the common plankters in the pelagic region of Cultus lake, as estimated from samples taken at one central station, and subject to the limitations of accuracy discussed above. It is based on stage catches with mesh 20 nets from 1923 to 1929, on total vertical hauls with mesh 20 net from 1932 to late 1933, on total vertical hauls with mesh 10 net from December 1933 to May 1934, and on stage catches with the plankton trap from June 1934 to June 1935. Only the last-mentioned section can be printed here (table I). The complete table is on file and may be referred to at the Pacific Biological Station, Nanaimo. Also reproduced here are charts (figures 1 and 2) showing the mean monthly abundance of the various organisms over the whole period. These are based upon the same observations as the table, except that the numbers of the adult Entomostraca from June 1934 to June 1935 are estimated from the hauls with no. 10 net, taken four times a month during that period. In addition, there has already been published (Ricker 1937c, p. 462) a table of the mean monthly abundance of Entomostraca during selected periods.

The distribution of the various genera of plankters may be discussed in turn:

ALGAE

Staurastrum. Occurs from late spring to early fall, only rarely exceeding 50 per litre. More than usually common in 1933, when the increase began in April and subsided in August.

Sphaerocystis. Midsummer to late fall; rather less common than the last.

Asterionella (figure 2). Spring pulse in most years, with as many as 10,000 colonies per litre, lasting from February to April or May; but very scarce in 1935. Fall pulse with as many as 1000 colonies in 1923, 1927, 1928 and 1932 (none in 1933 or 1934), lasting from late July to October or November.

TABLE I. Numbers of plankters taken from June 1934 to June 1935. The unit is centimetres of filament per litre in the case of *Melosira*, colonies per litre in the case of *Asterionella* and *Dinobryon*, and individuals per litre in all other cases. Each figure is the mean of

	1934											
	June		July		August			Sept.	October		November	
	4	16	4	17	1	16	31	14	1	17	2	18
Epischura	0.16	0.54	0.20	0.64	0.08	0.52	0.45	0.39	0.32	0.38	0.24	0.05
Cyclops	6.87	7.90	5.91	6.60	7.16	7.01	7.44	9.50	11.11	14.62	12.59	10.04
Nauplii	25.7	18.8	26.1	28.8	29.4	30.6	23.7	29.0	31.2	22.8	15.8	11.6
Daphnia	2.51	5.45	3.88	3.25	2.84	3.61	0.89	0.45	0.30	0.25	0.38	0.46
Bosmina	2.41	0.84	0.21	0.18	-	-	0.05	0.12	2.00	1.15	0.81	0.11
Conochilus	11.8	55.2	48.6	19.7	1.9	3.0	2.5	13.4	2.8	3.0	30.0	33.9
Asplanchna	2.09	0.50	0.46	0.76	0.94	0.34	0.32	0.37	0.56	0.32	0.22	0.08
Polyarthra	+	0.1	0.6	1.4	1.9	2.0	3.1	2.5	13.1	20.2	12.0	5.4
K. quadrata	+	-	-	-	+	-	-	+	0.2	+	0.4	+
K. cochlearis	-	-	-	-	0.1	-	0.1	0.2	0.5	0.1	0.3	0.5
Notholca	5.0	3.9	0.9	1.5	0.9	2.1	2.9	3.4	10.4	17.0	10.2	4.1
Synchaeta	0.1	0.1	0.2	-	-	+	+	0.1	0.1	-	0.1	-
Ploesoma	-	-	-	0.12	0.11	0.02	-	0.05	0.08	-	-	-
Dinobryon	+	-	0.1	1.0	7.5	116.0	269.0	75.6	26.0	26.2	0.6	0.2
Ceratium	1.1	1.4	17.7	16.9	43.1	60.7	87.1	107.0	71.5	10.3	1.9	0.1
Staurastrum	-	-	-	-	+	+	+	-	+	-	-	-
Sphaerocystis	-	-	-	+	+	+	+	+	+	+	-	-
Asterionella	+	-	-	-	+	-	+	-	-	-	-	-
Melosira	+	-	-	-	-	-	+	-	+	-	+	-
Synedra	-	-	-	-	-	-	-	-	-	-	-	-

Melosira (figure 2). Spring pulse each year of very varied proportions: up to 12 metres of filament per litre in 1932, and only 20 centimetres in 1925. Autumn pulse very poorly defined; a few filaments in some years. Spring pulse has a faint beginning in the preceding November, but is important only from February to April.

Synedra ulna. Of fairly regular occurrence in spring toward the close of the *Melosira* pulse in April and May; reaches about 500 individuals per litre.

PROTOZOA

Ceratium (figure 1). Summer and early fall, with a maximum usually in September of as many as 200 individuals per litre. Scarce in 1933.

9 counts made from samples taken at five-metre intervals over a depth of 40 metres, the top and bottom count being given half the weight of the others. A cross (+) indicates the occurrence of an organism in numbers too small to be enumerated by the technique used.

1935											
December			February		March		April		May		June
4	18	30	1	15	4	20	5	19	2	20	5
0.11	0.11	0.20	0.02	0.02	0.02	0.05	0.05	-	0.32	0.79	0.98
8.16	6.51	7.45	3.66	8.95	3.80	5.31	8.15	9.29	11.96	16.82	13.58
4.6	2.2	2.7	3.9	5.5	6.5	11.4	26.2	37.1	49.0	70.8	68.6
0.39	0.26	0.28	0.32	1.09	0.19	1.01	1.19	0.60	2.39	3.81	5.16
1.41	0.71	0.62	0.40	1.10	0.21	0.46	1.61	1.28	5.68	7.53	5.02
28.6	32.8	30.4	7.3	2.4	1.0	1.2	2.5	3.9	3.9	1.9	5.0
-	-	-	-	-	-	-	0.02	0.02	0.05	0.05	0.08
1.4	2.6	1.6	0.3	0.3	0.2	1.0	0.6	1.3	5.2	3.4	0.7
0.1	+	-	-	-	-	-	-	0.1	0.1	0.2	-
-	0.3	0.3	-	-	+	0.1	0.1	0.3	0.2	0.2	-
1.3	0.6	0.4	1.1	0.7	0.6	2.4	2.8	5.9	13.8	21.7	9.1
-	-	-	-	0.2	1.1	0.6	0.2	0.8	2.1	2.3	-
-	-	-	-	-	-	-	-	-	-	-	-
-	+	-	-	-	-	-	-	-	-	-	-
-	-	-	-	0.1	-	-	0.1	-	0.6	1.2	2.2
-	-	-	-	-	-	-	-	5.0	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	+	2.0	-	-	10.0	25.0	10.0	+	-
-	1.0	-	101.0	114.0	304.0	489.0	439.0	419.0	11.0	+	-
-	-	-	-	-	-	2.0	-	20.0	10.0	-	-

Dinobryon. Summer and fall, rather erratic, and subject to very sudden increase and decline in numbers. Up to several hundred colonies per litre in late August or early September of 1932 and 1934, scarce in 1933.

ROTATORIA

Conochilus (figure 1). Spring pulse culminates in June as a rule; there is a suggestion of a lesser one in August or September of 1925, 1933 and 1934; and a definite late-autumn pulse toward the end of November of 1933 and 1934. Scarce in 1932, and without well-marked trends. Recorded most abundantly in 1934, with over 50 per litre in spring and another large pulse in late autumn and early winter. The mixture of species probably contributes to the irregularity of the graphs of abundance.

Asplanchna (figure 1). Three years of abundance, 1925, 1928 and 1933, when it approached 20 per litre; and four of scarcity, 1929, 1932, 1934, 1935. Maxima in May or June; in "big" years it may be fairly common at all times.

Polyarthra (figure 1). Seasonal curve characteristically bimodal, with peaks in spring and fall. In 1928 and 1933 the spring maxima, with as many as 80

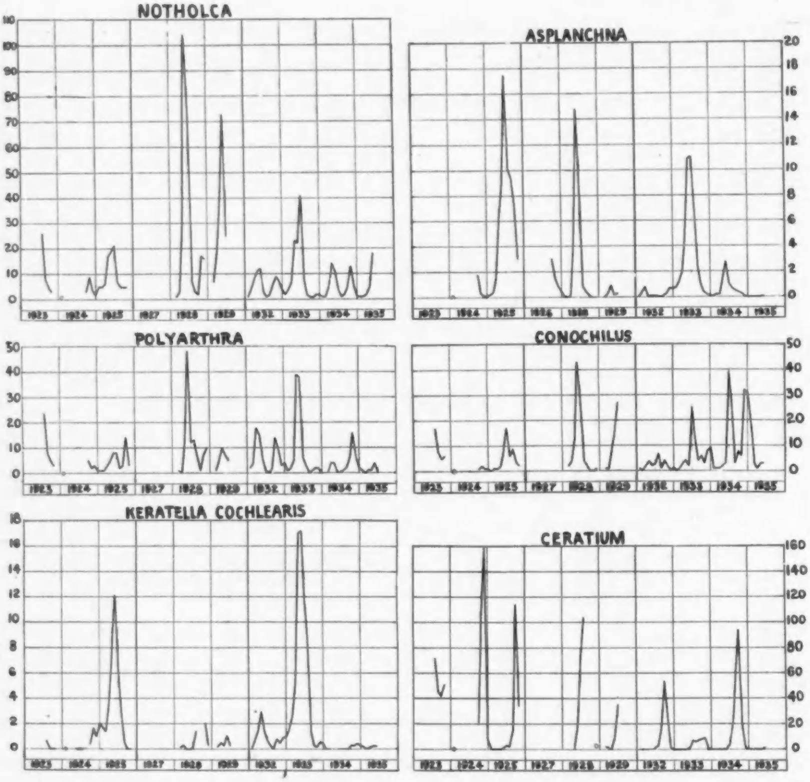

FIGURE 1. Fluctuations in abundance of plankters over ten years. *Notholca*, *Asplanchna*, *Polyarthra*, *Conochilus*, *Keratella cochlearis*, and *Ceratium*. Scale in individuals per litre.

per litre, greatly exceed those of the autumn, if indeed these last can be distinguished. In 1925, 1932 and probably 1934, the two were of the same order (20 per litre). Second in abundance, among rotifers, only to *Notholca*.

Keratella quadrata. Pronounced spring (April and May) pulse in 1932 (10 per litre), which is less evident in 1933, and in some of the earlier years. Suggestion of a fall pulse in each of 1932 and 1934.

Keratella cochlearis (figure 1). Two May peaks of some importance: 1925 and 1933, the last the larger with about 20 per litre. Much smaller peaks evident

in 1929 and 1932, doubtfully in 1928. Small fall pulses in 1924, 1928(?), 1932 and 1934.

Notholca (figure 1). May or June peaks in descending order: 1928, 1929, 1933, 1925, 1934 (?), 1932; ranging from 120 to 20 per litre. Definite October

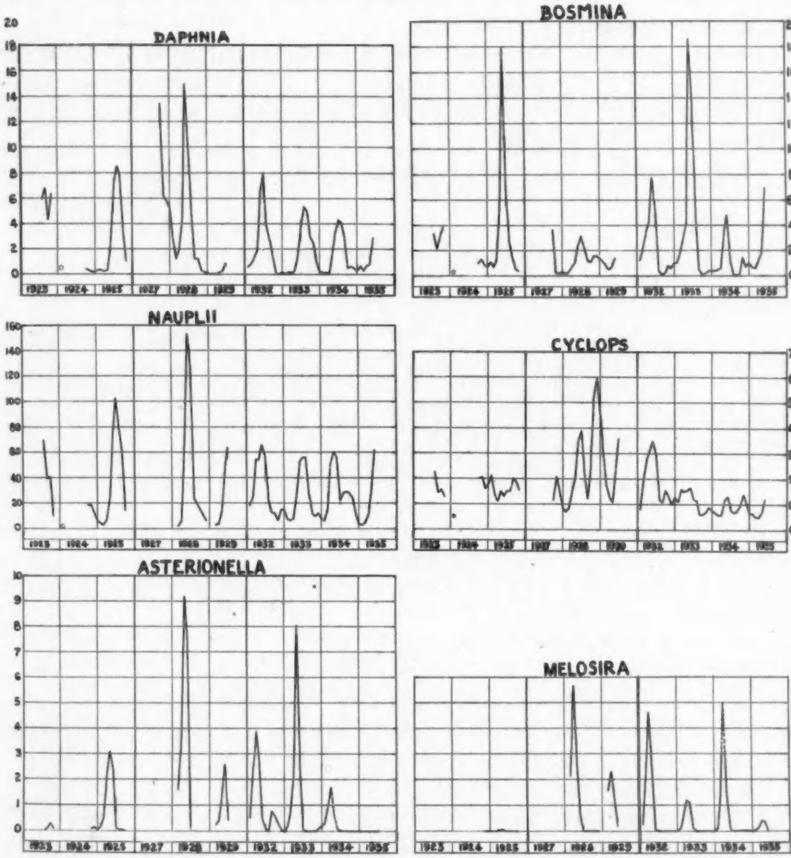

FIGURE 2. Fluctuations in abundance of plankters over ten years. Scale in metres of filaments per litre for *Melosira*, thousands of colonies per litre for *Asterionella*, and individuals per litre for *Daphnia*, *Bosmina*, *Cyclops* and the nauphlii.

peaks (10 to 20 per litre) in 1924, 1927, 1928, 1932, 1934. Abundant species, usually bimodal.

Synchaeta. March-May pulse in 1932 and 1933 with as many as 10 per litre; in 1935 the spring pulse appears to have two small peaks,—early March and mid-May. The apparent scarcity in earlier years may be the result of poor preservation.

Ploesoma. Recorded in only two years, 1927 and 1934. In the latter year it occurred from July to October, and its maximum abundance was 0.1 per litre.

ENTOMOSTRACA

Epischura. Summer form, commonest from July to September, when it may reach 0.8 per litre.

Cyclops (figure 2). Never less than 6 per litre, and up to 30 (1932) and possibly 60 (1928). Apparently of bimodal distribution, with peaks in spring and in autumn, but not definitely localized within these seasons. The spring maximum is usually the larger.

Copepod nauplii (figure 2) (undoubtedly mostly of *Cyclops*). Maxima in spring or early summer, with suggestion of a small fall maximum in some years. There is no apparent chronological relation between the abundance of the nauplii and that of the adult *Cyclops;* sometimes their peaks coincide, or either may follow the other. Nor is there good correlation apparent between the magnitude of a pulse of nauplii and that of adjacent pulses of the adult *Cyclops*.

Daphnia (figure 2). Exhibits pronounced unimodal fluctuation, with peaks from May to July. The time of subsidence of this summer pulse varies considerably; it is well down by September in some years (1925, 1928, 1932 and 1934), and in others lasts into late fall (1923, 1933), or even winter (1927). Peak numbers in recent years 5 to 9 per litre; in 1928 possibly up to 17. (It will be recalled that because *Daphnia* can avoid capture, these figures may be too low).

Bosmina (figure 2). Twenty per litre in May of 1925 and 1933, 11 in 1932, 8 in 1935, 6 in 1934, 3 in 1928, scarcely 2 in 1929, always declining abruptly in July. In years of small spring maxima, a smaller fall peak is evident, in October or November.

SUMMARY

The distribution of algae is characterized by seasonal pulses, either one or two per year, in which the various species increase to a maximum, then subside. The number occurring at the time of a maximum varies greatly from year to year; a species most numerous one season may be almost lacking in another. The species having two maxima (spring and fall) are the diatoms, the fall occurrence being much smaller and more irregular than the spring. The species having a single summer pulse are green algae (*Isokontae*) and blue-greens (*Myxophyceae*); only two species occur in any numbers, and they are much less common than the diatoms.

The two common protozoans each have a unimodal seasonal distribution, with peaks some time from summer to late fall, which are of very varied magnitude.

The rotifer genera may have unimodal, bimodal or possibly more complex seasonal distribution. Only one pulse per year has been observed for the large species of *Synchaeta*, *Asplanchna*, and *Ploesoma*. Bimodal distribution is characteristic of the common *Polyarthra* and *Notholca*, the spring pulse being usually the larger.

Of the Entomostraca, adult *Cyclops* are bimodal, with spring and fall pulses, but the numbers are subject to less extreme fluctuations than those of any other organism. *Epischura* and *Daphnia* have summer pulses, and are relatively scarce in winter. *Bosmina* is intermediate, its large pulse is in late spring, and a smaller fall peak sometimes occurs.

Causes of Fluctuations

The large fluctuations here observed from year to year in the numbers of organisms of several phyla of both plant and animal kingdoms are in general agreement with the findings of other limnologists, from Birge (1897) to the present day. On Cultus lake little success has been achieved in attempting to assess their causes however.

Pearsall (1923) was led to believe "that diatom periodicity is largely conditioned by floods, which affect the algae through their effects on the substances dissolved in the water . . .". Certainly the Cultus diatom maximum comes toward the end of the rainy season, but other considerations leave doubt as to the applicability of the above explanation. In January of 1935 there occurred exceptional rains which, with melted snow, raised the lake's level a metre overnight, and added a quantity of rubble unprecedented in recent years, to the deltas of the creeks entering the lake. Yet the pulse of *Melosira* in 1935 was with one exception the smallest on record, while *Asterionella* was scarce almost to the point of complete absence.

The fluctuations in abundance of certain mammals, birds and insects, over a period of years, have been shown to be cyclic. Among plankton crustaceans, Southern and Gardiner (1926) have demonstrated a type of periodic fluctuation in the case of the bimodal species *Daphnia longispina*, *Bosmina longirostris*, *B. coregoni* and *Diaptomus gracilis* occurring in Lough Derg. Over the two and one-half years studied, it was found that maxima could be grouped in pairs—two large ones followed by two small ones; these might be either autumn and spring of the same year, or of successive years, and there were three such arrangements among the four species mentioned. The Cultus lake data are unfortunately not yet complete enough to determine with certainty whether its plankters exhibit periodic oscillation in abundance, but none is readily apparent. The alternate large and small maxima of *Melosira* for the last four years are perhaps suggestive.

Climatic variability, expressed in the immediately evident temperature and certain other water characteristics, has not yet proved to be of value in accounting for differential abundance.

It is evident too that, when in any season an organism fails to appear in its usual abundance, there is no automatic development of another similar one to take its place in the lake's metabolism. On the contrary, the growth of both of two similar organisms is often checked, or stimulated, throughout the whole of one season. Comparing the two spring diatoms, in 1925 and 1933 *Asterionella* greatly exceeded *Melosira*, in 1928, 1929 and 1932 both species had a moderate to profuse development, in 1935 *Melosira* was scarce and *Asterionella* almost absent, leaving a gap which no other plant appeared to fill, unless it were some

nannoplankter. Among protozoans, both *Ceratium* and *Dinobryon* were abundant in 1932 and 1934, scarce in 1933. Considering the two abundant rotifers, it appears that the seasons of great abundance of *Notholca* usually coincide with those of *Polyarthra*, the spring of 1929 being the principal exception. No simple relationship of any kind is to be discovered between the years of abundance of the various entomostracans.

It is in respect to the relationship between the adult entomostracans and the sockeye salmon (*Oncorhynchus nerka*) that the most promising approach to date has been found. Juday and others (1932) have shown the effect of fertilization by decaying salmon carcasses upon the phytoplankton of certain Alaskan lakes. Cultus lake is fertilized in irregular fashion from year to year, owing not only to natural variation in the size of the sockeye runs, but also to their stoppage in years of artificial propagation. Some of the stripped carcasses have been carried, after spawning, to the upper part of the lake and thrown in. The number which passed into the lake in either of these ways is indicated below.

Year	Number entering lake naturally	Number transported
1925	5,420	none
1926	none	"
1927	81,000	"
1928	none	unknown—not more than 5,000
1929	none	about 1,500
1930	10,400	none
1931	19,000	about 7,000
1932	none	" 2,000
1933	"	none
1934	"	"

To these figures must be added several hundred coho (*O. kisutch*) each year, and an usually smaller number of chum salmon (*O. keta*).

Comparison of the quantity of various nutrient elements in dead sockeye with that occurring in other forms in the lake has been made in an earlier paper (Ricker 1937b), where it is shown that the decaying organic matter could be of significant importance to the larger lake animals only if it be used directly as it is broken up, rather than going through the long bacterial cycle which reduces it to inorganic salts, followed by reabsorption into phytoplankton. Various fish eat the decaying carcasses, and the possibility that plankters may also do so must receive consideration. The complete vertical water circulation of winter could distribute fine loose fragments of the decomposing fish throughout the whole lake, and various Cladocera have been observed by a number of students to feed and thrive on organic detritus and bacteria derived directly from it.

Granting the possibility of this effect, there is some suggestion of increased production of entomostracans in Cultus lake in years following a big escapement of sockeye into it, viz., 1928 and 1932. The increase is of *Cyclops* and *Daphnia*, but not *Bosmina*, which has greater peaks in other years. The relationship is only suggestive, but the plankton crop of 1936 will provide additional information.

Dry Weight and Nitrogen Content

In 1932 and in 1933 bimonthly total vertical hauls were taken with the Rawson nets. Those of the former year have been subjected to micro-Kjeldahl analysis for nitrogen content. It was hoped originally that this procedure would provide an accurate picture of the variations in quantity of macroplankton of all kinds, throughout the year. Unfortunately variations in efficiency of the net make the values of doubtful accuracy, especially as there are only a few determinations of its factor over the period in question. The data are however of some interest and are presented in figure 3 and table II. It will be noted that from late August to early February the quantity of net plankton, as indicated by its nitrogen content, is low: only about 2 mg. N_2 per cubic metre. It rises

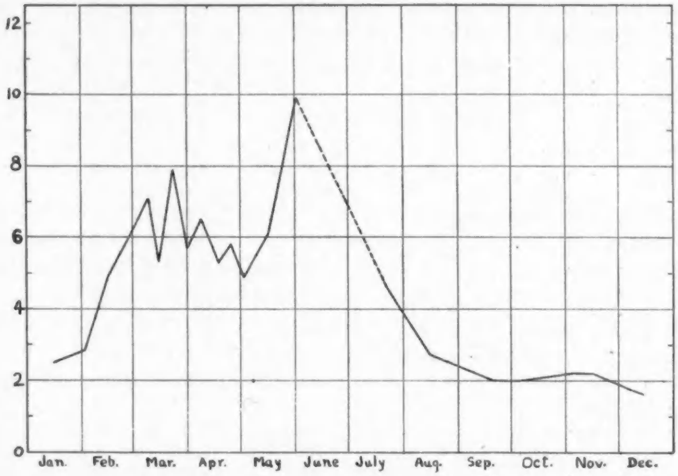

Figure 3. Variation in the total nitrogen content of the pelagic net plankton in 1932, expressed in milligrams per cubic metre.

rapidly with the pulse of diatoms in early spring, declines somewhat in April, then rises to 10 mg. in early June, coincident with the maximum of *Daphnia* at that time. Through the summer it falls off to the low autumn level.

Accurate comparison with the data of Rawson (1934) from Paul lake is not possible, but there is a suggestion that, in summer, that lake is about twice as rich in net plankton as is Cultus. Cultus lake spring values are however just as high as any that Rawson records in the period covered by his study (July to September).

The figures for dry weight of plankton, and the fraction of nitrogen in the dry weight shown in the table are of some interest. The weight follows much the same seasonal course as the nitrogen content, but is relatively greater in spring, when the siliceous skeletons of the diatoms contribute a large share.

A comparison of the plankton nitrogen with that existing in certain other organic and inorganic forms has already been made (Ricker 1937b).

TABLE II. Dry weight and total nitrogen content of the pelagic net plankton of Cultus lake, in 1932. To change dry weight and nitrogen content to read in kilograms per hectare of surface, multiply by 0.4.

Date 1932	Net factors (interpolated)	Dry weight (mg. /m²)	Nitrogen (mg. /m²)	Nitrogen (% of dry weight)	N_2 in sample (mg.)
Jan. 15	4.0	39	2.5	6.4	1.74
" "	"	54			
Feb. 2	4.8	112	3.3	2.9	1.92
" "	"	46	2.4	5.2	1.40
" 15	5.4	132	4.9	3.7	2.57
Mar. 2	5.8	186	6.4	3.4	3.13
" 9	6.2	191	7.1	3.7	3.23
" 15	6.5	232	5.3	2.3	2.29
" 23	6.7	225	7.9	3.5	3.33
" 31	6.9	146	5.7	3.9	2.32
Apr. 8	7.2	224	7.2	3.2	2.82
" "	"	150	5.8	3.9	2.28
" 18	7.4	138	5.3	3.8	2.02
" 25	7.5	111	6.0	5.4	2.24
" "	"	113	5.6	5.0	2.13
May 2	7.7	59	4.8	8.1	1.76
" "	"	70	5.0	7.1	1.83
" 16	7.8	77	5.8	7.5	2.12
" "	"	71	6.4	9.0	2.32
June 1	7.9	168	9.9	5.9	3.55
July 22	8.1	53	4.6	8.7	1.60
Aug. 15	8.1	43	2.5	5.8	0.87
" "	"	69	3.0	4.3	1.03
Sept. 20	8.2	32	2.0	6.2	0.69
Oct. 5	8.2	32	2.0	6.2	0.70
Nov. 4	8.3	33	2.2	6.7	0.76
" 16	8.3	27	2.3	8.5	0.78
" "	"	30	2.1	7.0	0.71
Dec. 2	8.3	26	1.7	6.5	0.58
" "	"	29	1.8	6.2	0.63
" 15	8.3	25	1.3	5.2	0.43
" "	"	29	1.9	6.5	0.64

SUMMARY

During 78 months extending over a period of 13 years samples of the net plankton of the open water of Cultus lake have been taken at a central station. Data are most complete for the period 1932-35, when collections were made semi-monthly or oftener.

A considerable variety of plants and animals were determined from the pelagic plankton, but the number of abundant genera is not great. They include two diatoms, two green algae, two protozoans, about eight rotifers, two copepods and two cladocerans.

The seasonal distribution of the various genera of plankters is of two principal types, unimodal (with a maximum in late spring or summer) and bimodal (with maxima in early spring and in autumn). Of the unimodal type are the green algae *Staurastrum*, *Sphaerocystis;* the protozoans *Ceratium*, *Dino-*

bryon; the rotifers *Asplanchna*, *Ploesoma;* and the entomostracans *Epischura*, *Daphnia* and *Bosmina* (the last sometimes having a small autumn mode as well). Of the bimodal type are the diatoms *Melosira*, *Asterionella;* the rotifers *Polyarthra*, *Keratella quadrata*, *K. cochlearis*, *Notholca*, *Synchaeta;* the entomostracan *Cyclops* and its nauplii. The rotifer genus *Conochilus* is irregular in its occurrence, owing probably to the two species involved. In the case of bimodal genera, the spring maximum is usually greater than the autumn one; the latter may indeed be barely perceptible, particularly with the diatoms.

Differences in abundance from year to year are characteristic of all the plankters studied. As a rule, in a year of an unusually large maximum population, the duration of a plankter's time of abundance is correspondingly increased. Data are not sufficient to say whether the observed fluctuations are cyclic, and they have not been correlated definitely with climatic or other factors.

During 1932 the total nitrogen content of the net plankton was determined. It had two maxima, of about 8 and 10 mg. per cubic metre, corresponding to the time of diatom and *Daphnia* pulses respectively. During autumn and winter it was fairly constant at about 2 mg. per cubic metre.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the close co-operation of Dr. R. E. Foerster in every part of this study, and particularly in placing at our disposal the plankton collections of years prior to 1932. We have also had the advice and assistance of Dr. W. A. Clemens, and of other members of the staff of the Pacific Biological Station, Nanaimo, B.C., and of staff and students of the Department of Biology, University of Toronto. Dr. E. H. Ahlstrom's kindness in identifying the organisms is greatly appreciated.

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The Feeding of Kingfishers: Food of Nestlings and Effect of Water Height

BY H. C. WHITE
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(*Received for publication March 9, 1938*)

ABSTRACT

Nestlings on the Margaree river, Nova Scotia, are fed young salmon and trout only, whose average size increases with that of the bird, indicating selection by the parents. The proportion of trout (*Salvelinus fontinalis*) to young salmon (*Salmo salar*) in the food of the adults varies directly with the height of the water.

The food of the kingfisher (*Megaceryle alcyon*) and other fish-eating birds on the Margaree river, Nova Scotia, has been investigated during the summers of 1935, 1936 and 1937. We have reported on the food and feeding habits of the adult and immature kingfishers, in relation to various habitats on the Margaree and Apple rivers (White 1936, 1937), but until this past year (1937) we had no data concerning the kinds and sizes of fishes fed to the nestlings by the parent birds.

FOOD OF NESTLINGS

During June and July, five broods of nestlings were secured from the upper part of the breeding range along the Northeast Margaree. These were preserved in formalin and retained for stomach analyses. We did not secure any newly-hatched nestlings, the youngest being about twelve days old. The nestlings which we secured weighed, after preservation in formalin, from 2.2. oz. to 5.8 oz. (1 oz. equals 28.3 grams) which weights are probably higher than they would have been before preservation.

Adult and immature kingfishers do not digest the bones of fishes but the nestlings dissolve the bones very quickly. Consequently, in the stomachs of the nestlings the only fishes which could be identified as to species, and whose lengths could be estimated, were those which had been recently ingested. Data concerning the analyses are given in table I.

The 33 nestlings contained identifiable remains of 34 salmon and 6 trout, which gives on a percentage basis 85 per cent salmon and 15 per cent trout.

SIZES OF FISHES

The sizes of all the salmon and trout found in the nestlings were estimated by comparing the bones with those from fishes of known lengths. These estima-

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tions are accurate within approximately one centimetre. In figure 1, the lengths of the fishes found in the stomachs have been plotted against the average weights of the nestlings. Since broods 1 and 2 averaged almost the same, as was also the case with broods 3 and 4, these pairs have been averaged together. In the figure the lengths of the fishes are plotted, using the numeral for the number of fishes of each length. A line has been drawn through the points representing the average lengths of fish found in the three lots of nestlings. This graph shows that the average fish fed to these nestlings increased with the weight of the nestling. There is considerable range in the sizes of the fishes found in the various broods. No fish over 10 cm. in length was found in the smallest nestling but no fish under 7 cm. was found in the largest nestling. Very small nestlings could not eat fish as large

TABLE I. Food analyses of kingfisher broods. Detailed analyses available from Fisheries Research Board of Canada.

Brood	Date	Place	Number nestlings	Average weight (oz.)	Salmon		Trout	Other stomach contents	
					1-year	2-year		Fish eyes	Chitin
1	1937 June 18	Black Rock pool	6#	2 1/6	3		2	Many	Much
2	June 26	Above Ingraham bridge	6	2 1/12	3		1	Many	Much
3	June 25	Below Ross' bridge	7	3 5/7	7	4	2	Many	Much
4	June 25	Below Ross' bridge	7	3 6/7	10		1	Many	Much
5	July 5	Marsh pool	7	5 6/7	3	3		Few	Little
Totals			33		26	7	6		

Seventh nestling retained for rearing.

as those which could be taken by the larger nestlings, but there is no apparent reason why the parent birds could not feed the smaller fishes to the larger nestlings. The data indicate a tendency for the parent birds to feed the young on fish approximating the limit in size which can be swallowed by the young.

SELECTIVE FEEDING

Brood 3 was found in a nest in the high bank of the flood plain. Below this bank was a long shallow lagoon containing an abundance of sticklebacks (*G. aculeatus*), a fish which is readily eaten by the older birds (White 1927, 1936, 1937). The young found in this nest contained eleven salmon parr and two trout, but no stickleback remains. In order to secure these salmon and trout for their young, the parent birds flew over the lagoon and brought fishes from the more distant river.

The nestlings of all broods contained salmon and trout only and these in the proportions of 85 per cent salmon and 15 per cent trout. Pellets and stomach analyses of adult and immature birds feeding along the same part of the river as that from which the nestlings were secured show that they had taken 90 per cent

salmon and trout, and 10 per cent other fishes (White 1936). The *relative* proportions of salmon and trout were 83 per cent salmon and 17 per cent trout, which is in close agreement with the percentages of these fishes found in the nestlings.

Since the adult and immature birds fed upon 90 per cent salmonids and 10 per cent other fishes, mostly sticklebacks, and the nestlings contained only salmonids, and since the parent birds of brood 3 did not utilize the abundant supply of sticklebacks in the nearby lagoon, it appears that the parent birds select certain fishes for their young.

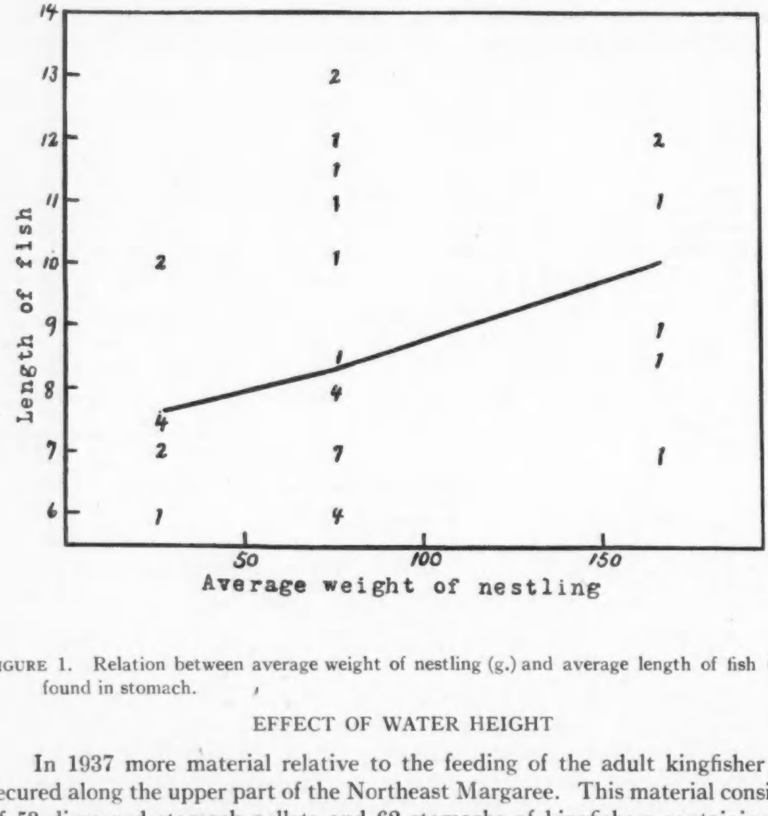

FIGURE 1. Relation between average weight of nestling (g.) and average length of fish (cm.) found in stomach.

EFFECT OF WATER HEIGHT

In 1937 more material relative to the feeding of the adult kingfisher was secured along the upper part of the Northeast Margaree. This material consisted of 53 disgorged stomach pellets and 62 stomachs of kingfishers containing the bones of 450 salmon, 214 trout (*S. fontinalis*), 19 sticklebacks (*Gasterosteus aculeatus*) and 1 water shrew.

EARLY FEEDING

At the time of the arrival of the kingfishers on the upper Margaree river in late April and early May (1937) there was still much snow in the woods, especially on the higher tablelands, and the melting of this snow kept the water levels of the

river and larger brooks high until the last of May. During this period we observed kingfishers feeding in small intervale spring-fed brooks and in the spring pools in the valley. Most of these places are well sheltered by alder bushes and contain an abundant supply of small trout, but young salmon are seldom found in such places. Since the kingfishers were feeding mostly in these small spring waters, pellets collected early in the season consisted largely of trout bones.

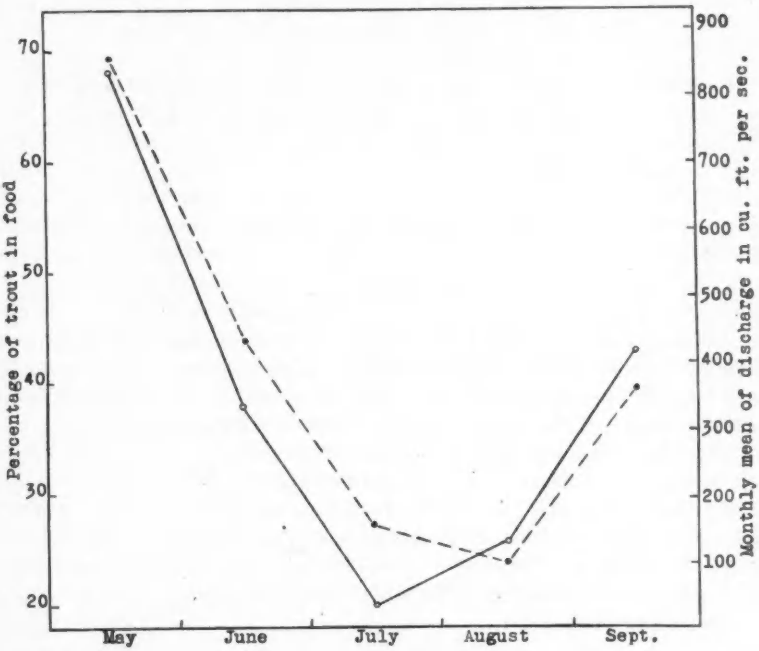

FIGURE 2. Relation of percentage of trout (continuous line) in kingfisher stomachs to mean monthly discharge of the Northeast Margaree river (interrupted line) in cu. ft. per sec. (1 = 1699.3 litres per min.).

LATER FEEDING

In the open reaches of the river and its tributaries young salmon are the dominant fish and these have been found to constitute the major part of the kingfisher's food in this region. When feeding upon these during normal water periods of the summer, kingfishers are found principally along the river or its larger tributaries. However, during high water they generally leave the larger waters and feed along the smaller brooks, even following these far into the thick woods. They are seldom found in such places during low-water periods.

Kingfishers shot during August and September, 1937, along Forest Glen brook, one of the larger tributaries of the Northeast Margaree, ordinarily contained a much higher percentage of salmon than of trout, but several birds shot

during high water in these months had more trout than salmon in their stomachs. These birds might have fed recently in one of the nearby spring brooks or might have secured the trout from the larger streams. Observations on the behaviour of trout and salmon in the larger waters have shown that during high water the trout generally remain nearer the surface than the salmon and thus even in waters where salmon are dominant the trout may, at times, be more available to kingfishers.

Water Height

Kingfisher pellets and stomachs collected during the various months showed the following percentages of trout: May, 68; June, 38; July, 20; August, 26; and September, 43. These analyses show a decline in the trout content until midsummer and then an increase.

At Frizzleton, a few miles (1 mi. equals 1.6 km.) below the area from which these data on the food were secured, daily readings of water level have been recorded by the Dominion Water Power Bureau. From these records the mean for the discharge in cubic feet per second has been calculated for each of the months of the period May to September and these plotted in figure 2. These data show declining discharge from May to August and an increased discharge in September. In the same figure, the percentage of the kingfishers' food which trout constituted is plotted for each month. A close correlation is to be seen between the changes in percentage of trout and the changes in water discharge.

In our previous studies of the kingfisher covering a variety of habitats in which were many species of fishes (White 1937, p. 332) we have concluded, "that the kingfisher feeds upon those fishes which are most available within its feeding range." The present observations for the upper Margaree where salmon parr and trout are the dominant fishes show that the relative availability of these two species varies with fluctuations in the water height.

AVAILABILITY OF DATA

Detailed tables giving the results of the analysis of the material used in the preparation of this paper may be obtained for reference from the Fisheries Research Board of Canada.

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Paphia bifurcata, a new molluscan species from Ladysmith Harbour, B.C.

By Daniel B. Quayle,
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(*Received for publication February 16, 1938*)

ABSTRACT

Paphia bifurcata: a new molluscan species from Ladysmith harbour, B.C. Distinguishing characters chiefly: shell shape; relatively shallow, rounded pallial sinus; and siphons separated at tips.

During the summer of 1937 the writer was engaged in oyster and clam work at Ladysmith, B.C., under Dr. C. R. Elsey of the Pacific Biological Station, Nanaimo, B.C. In the course of the investigation a few single valves of a clam, whose characteristics placed it in the genus *Paphia* (Bolten 1798) but were distinctive from *Paphia staminea*, were found among the empty shells of various mollusks. These shells differed markedly from *P. staminea* in shape, being proportionately less in height and diameter, and also in the pallial markings. After some search live specimens were found in mixtures of clean sand and fine shell along with *P. staminea* and *Saxidomus giganteus*. Examination of these specimens showed the siphons to be only partly fused, a very distinctive feature, hence the name *Paphia bifurcata* is assigned to this species here described as new to science.

Paphia bifurcata sp. nov.

Shell elongate, ovate, convex. Many radiating and concentric lines; radiating lines most prominent at anterior and posterior ends, but more so at latter, where short, blunt spines occur at the intersections. Hinge line very straight, ending abruptly over posterior adductor muscle and forming a definite angle with end of shell. Dorsal margin of shell anterior to umbo proportionately longer than in *P. staminea*, and anterior end sharply curved. Lunule long, narrow, coloration darker than surrounding shell, no radiating lines. Valves delicate, interiors smoothly polished and inner ventral margins not crenulate as in *P. staminea*. Pallial line well defined, regular; pallial sinus full, rounded, rising slightly toward umbo. Colour of valve varied, often with angular black markings; or mottled with gray, green, brown and black; or clear white. Hinge

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teeth not as heavy nor as prominent as those of *P. staminea*, but similar in number and arrangement. Internally mantle fusion similar to *P. staminea*. Incurrent and excurrent siphons not completely fused as in *P. staminea*, separated at tips, brown rather than black in colour, usually not as darkly coloured as those of the common species. Length 47 mm., height 35 mm., diameter 23 mm.

Type locality. Ladysmith harbour.

Range. Known only from the type locality, where it is relatively rare in comparison to the abundance of *P. staminea*.

Type specimen. Deposited in Pacific Biological Station.

Topotypes. Deposited at the University of British Columbia, Vancouver, the National Museum, Ottawa, the U.S. National Museum, Washington, the Department of Geology, Stanford University, Calif., and the Provincial Museum, Victoria, B.C.

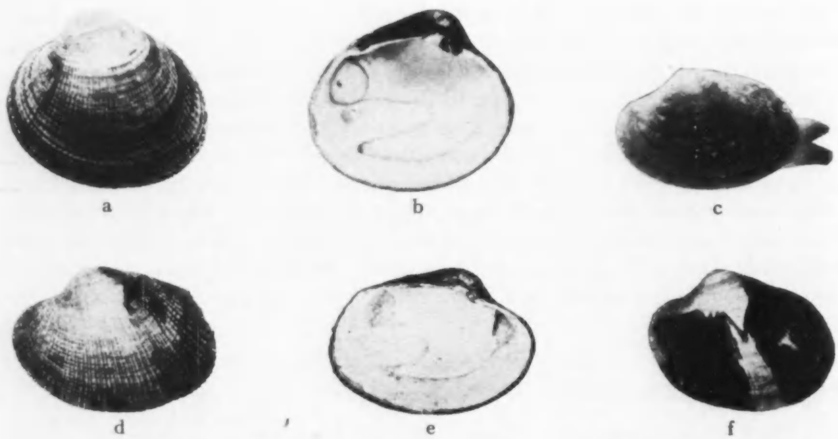

FIGURE 1. *Paphia staminea:* a,—left valve; b,—interior of left valve. *P. bifurcata:* c,—extended siphons; d,—left valve; e,—interior of left valve; f,—left valve showing angular black markings.

Fish Oils
VII. The Pigments of Pilchard Oil

By B. E. Bailey
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(*Received for publication February 22, 1938*)

ABSTRACT

Carotene, xanthophyll and fucoxanthin were found in commercial British Columbia pilchard oil, but only the two former in oil from canned fish. Heating commercial oil as in canning did not destroy the fucoxanthin, indicating its absence in the parts of fish canned.

The colour of pilchard oil varies from golden yellow to dark brown, with occasionally a distinct greenish tint. Tompkins (1930) states that this greenish colour is due to the presence in the oil of chlorophyll, derived from undigested plant material in the viscera of the fish. Biely and Chalmers (1936) have indicated that the yellow colour of commercial pilchard oil is probably produced by fucoxanthin.

The present work includes a qualitative study of the pilchard oil pigments, and quantitative estimations of three pigment fractions in three samples of British Columbia commercial pilchard oil and in two samples of oil prepared from canned British Columbia pilchards.

QUALITATIVE STUDIES

The pigments were first separated by the chromatographic method of Tswett (1906), which consists in passing a solution of the pigmented material through a vertical column of a suitable adsorbent and eluting the different pigments separately by virtue of their different adsorption affinities. This method has been described in full by Palmer (1922). In the present case a well-pigmented pilchard oil was dissolved in carbon disulphide and passed through a column of alumina. Part of the pigment was retained by the adsorbent, forming a dark orange-coloured band at the top of the column, while part did not appear to be adsorbed at all since the first liquid to come through had a yellow colour. The column was washed with pure carbon disulphide, only a small amount being necessary before the washings from the column became colourless. During this treatment the orange-coloured band at the top of the column spread out somewhat, but discrete bands did not appear. The column was next washed with benzene, which came through only very slightly coloured. Petroleum ether containing 1 per cent absolute alcohol was then run through the column. This caused two yellow bands to separate from the orange-coloured band at the top and to move down the column, one very rapidly but the other more slowly. The first of these was washed down to the bottom of

the column and eluted with this solvent. When it had been completely removed and clear washings were coming through, the more slowly moving band had still not progressed very far down. Washing with petroleum ether containing 10 per cent absolute alcohol quickly eluted the latter and caused the orange pigment still remaining at the top to move down the column. It was washed out and collected separately. The column was then free of pigment.

According to the description given by Palmer (1922) it appeared that the pigment which came through in carbon disulphide might be carotene, while the pigment eluted by petroleum ether containing 1 per cent alcohol and the first pigment eluted by petroleum ether containing 10 per cent alcohol appeared to be xanthophylls. Heilbron and Gillam (1937) have pointed out that, of the more important carotenoids, fucoxanthin is in general the most firmly adsorbed, so it appeared probable that the orange pigment finally eluted with petroleum ether containing 10 per cent alcohol might be fucoxanthin.

In order to obtain further evidence respecting the identity of these pigments, the partitions of the various fractions between immiscible solvents according to the methods of Willstätter and Page (1914) and Willstätter and Stoll (1913) were studied. Fucoxanthin is separated from the other pigments by the first of these methods, involving extraction of a solution of the pigments in equal parts of petroleum ether and ethyl ether with 70 per cent methyl alcohol. The second method utilizes the distribution between petroleum ether and higher concentrations of methyl alcohol to separate carotene and xanthophyll. Since most of the oil was contained in the fraction which had come through the chromatograph in carbon disulphide, only that fraction was saponified before studying these partitions between immiscible solvents. The unsaponifiable matter containing the pigments (supposedly carotene and xanthophyll) was separated from the soaps for examination by extracting the saponified mass, after dilution, with ethyl ether. The other pigment fractions which had been separated by chromatographic analysis were each transferred directly to solution in a mixture of equal parts of petroleum and ethyl ethers for the study of the phase partition, without previous saponification. All, including the fraction which was saponified, were examined by the same procedure.

The results of these partitions between immiscible solvents were as follows. The fraction which was not adsorbed at all yielded no fucoxanthin and only a small amount of xanthophyll, most of its colour being due to carotene. The fraction eluted with petroleum ether containing 1 per cent alcohol and the first fraction eluted with petroleum ether containing 10 per cent alcohol each yielded only xanthophyll, and the second fraction eluted by petroleum ether containing 10 per cent alcohol, only fucoxanthin.

The xanthophyll and fucoxanthin fractions thus isolated were transferred from their alcoholic solutions to ethyl ether. Each of these, as well as the solution of carotene in petroleum ether, was examined by the Deleano and Dick (1933) colorimetric test for carotene, and the colorimetric test for fucoxanthin described by Palmer (1922).

The fraction which from its previous behaviour appeared to be carotene gave a positive Deleano and Dick test but negative results with the Palmer reaction. The xanthophyll fractions gave negative results with both tests, while the fucoxanthin fraction gave a positive Palmer test but a negative Deleano and Dick test.

The evidence that carotene, xanthophyll and fucoxanthin were present was thus fairly conclusive. Although three carotenes and several xanthophylls are known, no further fractionation of any of these fractions was attempted.

QUANTITATIVE STUDIES

Quantitative separations of the carotene, xanthophyll and fucoxanthin in the five samples of pilchard oil were made by partition between immiscible solvents. Twenty-five grams of the oil was dissolved in 50 ml. of ethyl ether and 50 ml. of petroleum ether and the fucoxanthin extracted by the method of Willstätter and Page (1914). After removal of the fucoxanthin the solvent was distilled off and the oil saponified by refluxing with 60 ml. of 20 per cent colourless alcoholic potassium hydroxide. The saponified mass was diluted with three volumes of distilled water, and the unsaponifiable matter separated by two extractions with ethyl ether. After thoroughly washing and dehydrating the ethereal extract, the solvent was distilled off and the residue taken up in petroleum ether. The xanthophyll fraction was separated from the carotene by the method of Willstätter and Stoll (1913). The carotene, which remained in the petroleum ether, was diluted by further additions of that solvent to a definite volume and the depth of colour measured in a Lovibond tintometer. The concentration of carotene was calculated from the data thus obtained by the method of Ferguson (1935). The fucoxanthin and xanthophyll were transferred from their solutions in alcohol to ethyl ether. These ethereal solutions were compared with a potassium dichromate standard in a Duboscq colorimeter, and the pigment concentrations calculated by means of the formula given by Palmer (1922).

The results are presented in the following table:

Sample	Carotene mg. per 100 g. oil	Xanthophyll mg. per 100 g. oil	Fucoxanthin mg. per 100 g. oil
1935 commercial pilchard oil	0.06	0.49	0.16
1936 commercial pilchard oil—A	0.17	0.66	0.48
1936 commercial pilchard oil—B	0.25	0.84	0.84
Oil from canned pilchards—A	0.02	0.61	0.00
Oil from canned pilchards—B	0.13	0.64	0.00

As can be seen from the table, there was no fucoxanthin present in either of the samples of oil prepared from canned pilchards, although it was found in all the samples of commercial pilchard oil. It was thus evident that either fucoxanthin was not present in the parts of the fish which were canned, or that it was destroyed in the canning process. In order to determine which was the case, some commercial pilchard oil was sealed in a can and subjected to the regular canning process (90

minutes' cooking at 115° C.). Fucoxanthin was found to be still present in this oil after the heating, so it would thus appear that the fucoxanthin in the commercial pilchard oils was derived from the viscera, or visceral contents, of the fish.

SUMMARY

The presence of carotene, xanthophyll and fucoxanthin in British Columbia pilchard oil was indicated. A quantitative study of these pigments in three samples of the commercial oil gave values for carotene ranging from 0.06 to 0.25, for xanthophyll from 0.49 to 0.84 and for fucoxanthin from 0.16 to 0.84 mg. per 100 g. of oil. Two samples of the oil from British Columbia canned pilchards contained respectively 0.02 and 0.13 mg. of carotene, and 0.61 and 0.64 mg. of xanthophyll per 100 g. of oil, but no fucoxanthin. The fucoxanthin in a sample of the commercial pilchard oil was not destroyed by heating the oil to 115° C. for 90 minutes, indicating that this pigment is probably not present in the parts of the fish canned.

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Fish Oils

VIII. The Approximate Composition of the Fatty Acids of the Oil of Pilchards (Sardinops caerulea)

By H. N. Brocklesby and K. F. Harding
Pacific Fisheries Experimental Station

(*Received for publication February 22, 1938*)

ABSTRACT

Pilchard oil contains approximately 23% saturated fatty acids and over 15% highly unsaturated C_{24} acids. Unsaturated C_{16}, C_{18}, C_{20} and C_{24} acids are also present in considerable amount.

In the course of the past ten years the production of pilchard (California sardine) oil has assumed an important position in the fishing industry of British Columbia. During this time several reports have been issued from these laboratories concerning the properties and commercial uses of this oil and during these technical researches considerable information has been obtained regarding the composition of pilchard oil fatty acids. The present paper reports a complete analysis of these acids by the methyl ester fractionation method. The preceding paper in this series dealt with the pigments present in the oil and a subsequent paper will consider other non-saponifiable constituents.

The composition of pilchard oil fatty acids as found in a sample produced commercially during July, 1935, from whole pilchards is shown in table I. The

Table I. Approximate composition of Canadian pilchard oil fatty acids.

Acids	Solid acids "S" %	Liquid unsat'd acids, "U" %	Liquid highly unsat'd acids "HU" %	Total %	Neut. equiv. of acids	Unsaturation as –H per mole
Saturated						
Myristic (C_{14})	4.91	0.18		5.09	228.2	
Palmitic (C_{16})	13.90	0.48		14.38	256.3	
Stearic (C_{18})	3.19			3.19	284.3	
Unsaturated						
C_{14}		0.07		0.07	226.2	2.00
C_{16}	3.05	8.69		11.74	254.3	2.00
C_{18}	2.20	13.73	1.74	17.67	281.1	3.29
C_{20}	1.24	5.83	10.81	17.88	308.3	4.12
C_{22}			13.80	13.80	332.0	8.47
C_{24}			15.24	15.24	357.6	10.90

figures under solid acids "S" refer to the fatty acids whose lead salts are insoluble in 95 per cent ethyl alcohol at room temperature. The liquid unsaturated acids "U" and the liquid highly unsaturated acids "HU" refer to the acids whose lithium salts are respectively insoluble and soluble in 95 per cent acetone. An inspection of the data shows that, of the total fatty acids, approximately 23 per cent are

TABLE II. Analytical data of final methyl ester fractions.

Fraction†	Corrected weight (grams)	Sap. equivalent	Iodine value	Sap. equivalent saturated acids
Esters of saturated fraction "S" 28.5% of total acids				
S-1	4.87	250.5	12.3	231.7
S-2	10.17	252.3	12.4	241.9
S-3	14.19	267.9	12.5	254.4
S-4	7.81	268.9	13.6	256.0
S-5	7.90	272.2	17.8	259.6
S-6	9.15	287.0	36.7	269.1
S-7	6.00	307.3	69.2	281.2
Esters of unsaturated fraction "U" 29.0% of total acids				
U-1-1	3.94	265.0	80.2	250.0
U-1-2	5.74	267.2	81.9	261.8
U-2	8.60	277.8	112.6	270.3
U-3	11.58	284.3	119.6	....
U-4	6.47	290.2	123.7	....
U-5-1	4.14	291.5	138.0	....
U-5-2	4.02	292.8	147.0	....
U-5-3	1.88	299.8	155.9	....
U-5-4	4.27	302.2	156.9	....
U-6-1	4.60	314.2	213.2	....
U-6-2	4.99	316.1	224.0	....
U-6-3	2.36	319.2	229.3	....
U-6-4	4.89	326.5	223.8	....
Esters of unsaturated fraction "HU" 41.6% of total acids				
HU-1-1	6.90	306.3	245.3	....
HU-1-2	12.98	322.2	278.3	....
HU-2-1	8.17	332.2	283.1	....
HU-2-2	9.82	344.8	313.5	....
HU-2-3	3.54	350.5	339.4	....
HU-2-4	4.45	352.1	339.1	....
HU-3-1	3.30	354.2	330.4	....
HU-3-2	6.68	358.8	340.6	....
HU-3-3	3.52	361.7	344.0	....
HU-3-4	6.31	368.2	335.5	....
HU-4	4.90	360.0	348.7	....
HU-5	11.21	371.3*	338.4	....

*Corrected saponification equivalent after removal of 1.54 per cent unsaponifiable matter.

†The first figures indicate primary fractions. Second figures indicate refractionations of the primary fractions.

saturated, palmitic acid being present in considerable excess over myristic and stearic. These figures agree fairly well with those found by Armstrong and Allan (1924, p. 216T) for the oils from whole fish of the related species, *Clupanodon melanostica* (Japanese sardine) and *Alosa menhaden* (menhaden), and by Lovern (1932) for *Clupea spratus* (sprat).

In regard to the unsaturated fatty acids, however, the agreement between the present data for pilchard oil and for the related oils quoted above is not as good. The chief difference lies in the occurrence in pilchard oil of considerable quantities of C_{24} acids of high unsaturation which according to the above authors are not present in the oils investigated by them. In the comprehensive summary of the fatty acid composition of fish oils given by Hilditch in Schönfeld (1936, pp. 90-96) the liver oils of the elasmobranchs, family *Squalidae,* appear to be the only examples containing C_{24} acids but in these cases the average unsaturation is only –2H to –3H.

The present data are corroborated by the work of Toyama and Tsuchiya (1935) who have isolated from Japanese sardine oil an acid (nisinic) with the formula $C_{24}H_{36}O_2$. Indication of the presence of acids with the formulae $C_{24}H_{38}O_2$ and $C_{24}H_{40}O_2$ were also obtained by these workers. Unfortunately a recent complete quantitative assay of Japanese sardine oil fatty acids is not available for comparison with that of pilchard oil.

As reported in another communication (Brocklesby and Denstedt 1933, p. 367) the characteristics of pilchard oil undergo considerable seasonal changes and it is planned to follow these changes by methyl ester fractionations.

EXPERIMENTAL

The analytical characteristics of the pilchard oil used were as follows: iodine value 183.9, saponification value 198.8, refractive index at 25° C. 1.4794.

Five hundred grams of the oil were saponified with boiling alcoholic potash. After dilution with water the unsaponified material was removed with ethyl ether. The residue after evaporation of the ether solution was resaponified and washed again with ether. The second aqueous fraction was added to the original ether-washed soaps and the fatty acids recovered. The unsaponifiable material amounted to 6.15 grams. In all cases removal of solvents was accomplished at reduced pressure in an atmosphere of hydrogen.

Four hundred grams of the free fatty acids were treated with lithium hydroxide in acetone by Tsujimoto's method (1920, p. 1007) when 166.6 grams of fatty acids from the acetone-soluble lithium salts were recovered. These were immediately analysed, methylated and fractionally distilled. Two hundred and nineteen grams of fatty acids from the acetone-insoluble lithium salts were subjected to the lead salt-alcohol separation and the solid (108.1 g.) and liquid (109.7 g.) acids isolated and methylated. The latter operation was carried out by refluxing with absolute alcohol saturated with dry hydrogen chloride. The recovered esters were washed with water, saturated sodium bi-carbonate solution, and again with water until neutral. They were then dried and fractionally distilled.

All distillations were made in a modified form of the G.L.C. (Gooderham

1935) fractionating column in which the vacuum jacket is replaced by a heavily lagged heating element in series with that which heats the boiling tube. The modified column also carries a Claisen head which facilitates the use of a hydrogen bubbling tube. This modified apparatus has been found to be very efficient in methyl ester fractionation, for which purpose it is constructed in three sizes, a large size (boiling tube capacity 300 ml.) for the primary fractionations, and two smaller sizes (150 and 50 ml.) for refractionations. The column is used in conjunction with the vacuum receiver described previously (Brocklesby and Denstedt 1931, p. 367).

Primary distillations were carried out at pressures of 0.45 to 0.75 mm. Hg. and secondary distillations at pressures of 0.1 to 0.3 mm. Hg. The highly unsaturated esters were fractionated, refractionated and analysed within a few days after the initial lithium salt separation. All esters were stored under nitrogen at –20° C. until fractionated and/or analysed. Saturated acids were isolated from the methyl ester fractions by Bertram's method as modified by Hilditch and Priestman (1931). Unsaturation was determined by the Wijs method and in the final table of composition the average unsaturation has been reported in terms of –H per mole of fatty acid.

The calculations of compositions of the fractions were made, in general, by the methods outlined by Charnley (1934). All but two of the saturated fatty acid-free fractions were found to be binary mixtures. Two fractions (U-5-2 and HU-3-2) were ternary. The composition of these was solved by extrapolation of the molar concentrations of the component acids in the adjacent fractions. In order to conserve space the data for the final fractions only are given in table II. The weights of these final fractions are corrected to take into account material used for analysis in the primary fractions. The composition of the fatty acids given in table I are calculated directly from the data in table II in conjunction with those given for the lithium and lead salt separations.

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